Marine Pollution Bulletin 121 (2017) 222–229

Contents lists available at ScienceDirect

Marine Pollution Bulletin

journal homepage: www.elsevier.com/locate/marpolbul

Plastic ingestion in oceanic-stage loggerhead sea turtles (*Caretta caretta*) off the North Atlantic subtropical gyre

Christopher K. Pham[a,*], Yasmina Rodríguez[a], Axelle Dauphin[b], Rita Carriço[a], João P.G.L. Frias[a], Frederic Vandeperre[a], Vanessa Otero[c], Marco R. Santos[d], Helen R. Martins[a], Alan B. Bolten[e], Karen A. Bjorndal[e]

[a] IMAR-Institute of Marine Research and MARE—Marine and Environmental Sciences Centre, University of the Azores, Horta, Portugal
[b] ENVT - École Nationale Vétérinaire de Toulouse, 23 Chemin des Capelles, 31300 Toulouse, France
[c] Department of Conservation and Restoration and LAQV-REQUIMTE, Faculdade de Ciências e Tecnologia da Universidade Nova de Lisboa, 2829-516 Monte da Caparica, Portugal
[d] DRAM - Direção Regional dos Assuntos do Mar/Regional Directorate for Sea Affairs, Secretaria Regional do Mar, Ciência e Tecnologia, Colónia Alemã – Apartado 9, 9900-014 Horta, Faial, Portugal
[e] ACCSTR - Archie Carr Center for Sea Turtle Research, Department of Biology, PO Box 118525, University of Florida, Gainesville, FL, USA

ARTICLE INFO

Keywords:
Plastic
Sea turtles
Caretta caretta
Azores
Oceanic-stage
North-Atlantic subtropical gyre

ABSTRACT

Juvenile oceanic-stage sea turtles are particularly vulnerable to the increasing quantity of plastic coming into the oceans. In this study, we analysed the gastrointestinal tracts of 24 juvenile oceanic-stage loggerheads (*Caretta caretta*) collected off the North Atlantic subtropical gyre, in the Azores region, a key feeding ground for juvenile loggerheads. Twenty individuals were found to have ingested marine debris (83%), composed exclusively of plastic items (primarily polyethylene and polypropylene) identified by μ-Fourier Transform Infrared Spectroscopy. Large microplastics (1–5 mm) represented 25% of the total number of debris and were found in 58% of the individuals sampled. Average number of items was 15.83 ± 6.09 (± SE) per individual, corresponding to a mean dry mass of 1.07 ± 0.41 g. The results of this study demonstrate that plastic pollution acts as another stressor for this critical life stage of loggerhead turtles in the North Atlantic.

1. Introduction

The amount of plastic entering the world's oceans is estimated to be 4–12 million tons annually (Jambeck et al., 2015), making it the principal component of marine debris. With its persistent nature, light weight, and great dispersal capabilities, plastic has rapidly been recognized as a global environmental threat that severely affects marine ecosystems (Bergmann et al., 2015).

Ingestion and entanglement in plastic debris has been reported for a wide variety of organisms, from small zooplankton to baleen whales (Kühn et al., 2015). So far, > 700 species have been reported to ingest marine plastics (Gall and Thompson, 2015) and the number of occurrences is constantly increasing. In some areas, entire populations are at risk (e.g. Knowlton et al., 2012; Richards and Beger, 2011) with cascading effects that may eventually result in the disruption of key ecosystem function and services (Newman et al., 2015).

The complex life histories, highly mobile behavior and feeding ecology of sea turtles makes them particularly vulnerable to plastic pollution, especially smaller, oceanic-stages (Schuyler et al., 2014a). Together with the widespread distribution of plastic in the marine environment, ingestion of and entanglement in plastic debris have inevitably become one of the most important threats for sea turtle populations worldwide, with all seven species reported to be affected (Nelms et al., 2016). The probability of interactions between sea turtles and plastic is directly linked to the feeding ecology and habitat use of the species and/or life stages and to the spatial distribution of plastic in the marine environment. Therefore, the threats caused by plastic pollution differ significantly between species, populations and life stages (Schuyler et al., 2014a).

Sea turtles may suffer lethal and sub-lethal effects when a plastic item is mistaken for food (McCauley and Bjorndal, 1999) or when debris is mixed with natural prey (Di Beneditto and Awabdi, 2014). The consequences from the ingestion of anthropogenic items for sea turtles can be dramatic and includes internal injuries and intestinal blockage, interference with swimming behavior and buoyancy, or accumulation of plasticizers or heavy metals and other toxins, such as PCBs (see

* Corresponding author.
E-mail address: phamchristopher@gmail.com (C.K. Pham).

http://dx.doi.org/10.1016/j.marpolbul.2017.06.008
Received 23 April 2017; Received in revised form 2 June 2017; Accepted 3 June 2017
Available online 09 June 2017
0025-326X/ © 2017 Elsevier Ltd. All rights reserved.

Nelms et al., 2016 for a recent review). Although a global awareness on the impacts of marine debris in sea turtle populations has increased in the last decades, intensive monitoring programs are imperative to quantify the true scale of the problem and provide a baseline necessary to evaluate the efficacy of upcoming public policies aimed at reducing plastic input into our oceans.

Loggerhead turtles (*Caretta caretta*) are the most common sea turtle species occurring in the Azores, originating mainly from rookeries of the east coast of North America (Bolten et al., 1998). Following their journey across the North Atlantic subtropical gyre, juveniles stay around the Azores ~7–12 years (Bjorndal et al., 2003), feeding predominantly on planktonic and neustonic organisms (Frick et al., 2009). Oceanic stage loggerheads in the region show a strong association with the seamounts that are abundant in the area, probably for feeding and navigation (Santos et al., 2007).

Throughout their distribution, loggerhead turtles have been reported to ingest debris (Bugoni et al., 2001; Limpus et al., 2001; Tomás et al., 2002; Parker et al., 2005; Casale et al., 2008; Lazar and Gračan, 2011; Campani et al., 2013; Camedda et al., 2014; Hoarau et al., 2014; Casale et al., 2016; Nicolau et al., 2016). Their wide distribution across the Atlantic Ocean and Mediterranean Sea, together with their susceptibility to ingest marine debris, makes them adequate indicators for monitoring plastic pollution in the oceans. As a result, loggerheads were recently proposed as indicator species for Descriptor 10 of the European Union's Marine Strategy Framework Directive (MSFD) ("indicator 10.2.1", Galgani et al., 2014).

The objective of this study was to quantify debris ingestion in oceanic-stage loggerhead turtles in the Azores Archipelago, located at the fringe of the North Atlantic subtropical gyre which is considered a hotspot for floating debris (Law et al., 2010). The ingestion of marine debris by oceanic-stage loggerheads in the North Atlantic was previously addressed by Frick et al. (2009) when studying their feeding ecology. However, debris ingestion was not the primary goal and more data are needed in order to fully assess the current threat of marine debris for juvenile oceanic-stage loggerhead turtles.

2. Materials and methods

2.1. Study area

The material analysed in this study was collected in an area located at the northern edge of the North Atlantic Subtropical Gyre, in the Azores region (Fig. 1). The Azores is a Portuguese archipelago composed of nine islands situated on the Mid-Atlantic Ridge with an extensive exclusive economic zone (EEZ) of about 1 million km^2 (Fig. 1). The islands are of volcanic origin and are characterized by narrow shelves and steep slopes. The surrounding waters have an average depth of 3000 m with only < 1% of the total EEZ being shallower than 600 m (Perán et al., 2016). Seamounts are common features in the Azores and may occupy 37% of the total area of the EEZ (Morato et al., 2008, 2013).

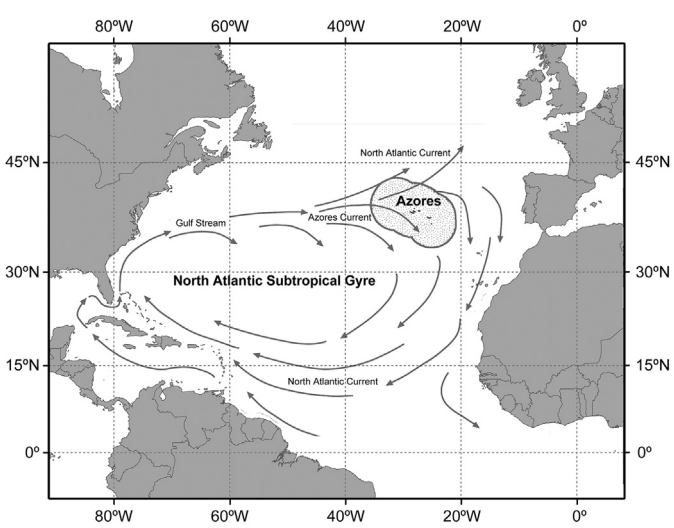

Fig. 1. Schematic of the North Atlantic Subtropical Gyre, the Azores archipelago and its exclusive economic zone (EEZ) of ~1 million km^2. Arrows show the generalized direction of current flow.

2.2. Sample and data collection

The material analysed in the present study was collected between 1996 and 2016 from dead turtles found stranded along the coast, floating dead, or accidentally caught by surface longline gear (Table 1). Entire animals, individual organs or contents were either frozen at −20 °C, or preserved in formaldehyde or ethanol solutions for later analysis. Before performing the necropsies, individuals were weighed and measured to the nearest millimetre. After obtaining these measurements, animals were opened and each organ was carefully examined. The entire gut was divided into three sections (oesophagus, stomach and intestines) and separated with the help of small strings. For some individuals, not all organs could be preserved and analysed (see Table 1). Individual organs were weighed and their contents filtered using a 1 mm sieve. The material was placed in a petri dish/container with clean water. Each plastic item rose to the surface and was carefully collected.

All pieces of debris were counted, weighed (dry mass), measured (maximum length) and described with the highest level of detail possible. Following the suggestion made by the MSFD "Guidance on Monitoring of Marine Litter in European Seas" (Galgani et al., 2013), each item was eventually classified following van Franeker et al. (2011) into: (1) industrial plastic (pellets) and (2) user plastic (sheet, thread, foam, fragments, other). In addition, we included another category for fishing-related plastics (nylons, ropes and conglomerates of fishing lines). Ultimately, every anthropogenic item was associated with a colour (white, transparent, yellow, blue, green, black, grey, brown, red, pink and orange). Multi-coloured items were put into the category "coloured". Items 1–5 mm were referred as "large microplastics", items between 5.1 and 25 mm as "mesoplastics" and above 25 mm as "macroplastics". Organic items such as wood and feathers were catalogued as 'natural debris' and were not considered in our general analysis of debris ingestion, as these items do not likely come from anthropogenic sources.

2.3. Data analysis

The amount of items ingested (both in terms of number of items and dry mass) was computed as 'population averages' (with standard error of the mean), which includes both individuals that ingested and did not ingest plastics, as recommended by Kühn et al. (2015). Because normality assumptions were not met, Kruskal-Wallis-H tests (non-parametric one way analyses of variance) were performed to evaluate the differences in number and dry mass of plastic items ingested by turtles between different size classes and types of organs. Sea turtles were binned in size classes of 10 cm: 1–11; 11.1–21; 21.1–31; 31.1–41; 41.1–51; 51.1–61; 61.1–71 cm (CCL). In addition, for comparisons with other studies on debris ingestion in loggerheads, turtles were reclassified into two groups according to size: small (CCL ≤ 40 cm) and large (CCL > 40 cm) juveniles (Camedda et al., 2014; Nicolau et al., 2016). Spearman's rank correlation coefficient was used to examine the relationship between loggerhead size classes and maximum debris length.

2.4. Plastic identification

Plastics retrieved from the different organs sampled were

Table 1
Collection and sampling information along with details on plastic ingestion for the 24 loggerhead sea turtles (*Caretta caretta*) sampled in the Azores.

Tags	Year	Source	CCL (cm)	Total n° of debris	Total dry mass of debris (g)	Oesophagus		Stomach		Intestines	
						n°	Dry mass (g)	*n*°	Dry mass (g)	*n*°	Dry mass (g)
T1	1996	Stranded dead	71.0	9	2.11	–	–	–	–	9	2.11
P7549	2000	Bycatch	42.7	19	0.91	–	–	–	–	19	0.91
P8301	2000	Bycatch	50.5	42	0.62	–	–	–	–	42	0.62
P8475	2000	Stranded alive[b]	24.3	2	0.09	–	–	0	0.00	2	0.09
P8051	2001	Bycatch	32.2	139	5.94	–	–	–	–	139	5.94
P8073	2001	Bycatch	56.0	7	0.14	–	–	–	–	7	0.14
P8567	2004	Bycatch	57.4	55	5.01	0	0.00	14	0.70	41	4.31
P8596	2004	Bycatch	34.7	32	1.36	0	0.00	32	1.36	–	–
P9451	2004	–	26.2	0	0.00	–	–	0	0.00	–	–
P9456	2004	Stranded alive[b]	12.0	3	0.03	–	–	–	–	3	0.03
P9453	2006	Stranded dead	9.4	0	0.00	–	–	–	–	0	0.00
P9455	2007	Stranded dead	13.4	7	0.13	–	–	–	–	7	0.13
P9454	2008	Floating dead	16.3	18	0.53	–	–	18	0.53	–	–
P9644	2009	Floating dead	20.2	1	0.01	–	–	0	0.00	1	0.01
N8431	2010	Stranded dead	10.9	9	0.18	–	–	–	–	9	0.18
PA148	2011	–	57.4	4	0.02	0	0.00	0	0.00	4	0.02
HJ4055	2013	–	55.6	2	0.01	0	0.00	2	0.01	0	0.00
PA147[a]	2013	Stranded dead	60.5	16	7.09	9	2.75	4	3.12	3	1.22
N8546	2016	Stranded dead	12.5	0	0.00	0	0.00	0	0.00	0	0.00
N8548	2016	Stranded dead	10.5	1	0.01	0	0.00	0	0.00	1	0.01
P7364	2016	Stranded dead	10.0	0	0.00	0	0.00	0	0.00	0	0.00
HJ4052	2016	Stranded dead	11.5	1	0.03	0	0.00	1	0.03	0	0.00
HJ4058	2016	Stranded dead	35.5	12	1.53	0	0.00	6	1.53	6	0.00
HJ4056	–	–	45.9	1	0.02	0	0.00	1	0.02	0	0.00

[a] Turtle with hook stuck in the oesophagus.
[b] Later died.

characterized by micro-Fourier Transform Infrared Spectroscopy (μ-FTIR) in order to identify common polymers. A composite sample was selected that represents the main types of debris recovered. This composite of 23 samples (6% of the total number of items) included plastic fragments, sheets, ropes and pellets.

The μ-FTIR analysis was carried out as described in Frias et al., 2014, at the Department of Conservation and Restoration (DCR) of the Universidade Nova de Lisboa. Micro-samples were carefully cut under a Leica KL 1500 LCD microscope, equipped with a 12× objective and a Leica® Degilux 1 digital camera, with external illumination by optical fibres. Samples were compressed in a *Thermo* diamond anvil compression cell, and infrared spectra were acquired in a Nicolet® Nexus spectrophotometer coupled to a Continuμm microscope (15× objective) with a MCT-A detector cooled by liquid nitrogen. Spectra were collected in transmission mode, 4000–650 cm^{-1}, with a resolution of 4 cm^{-1} and 128 scans.

The spectra are shown as acquired, without corrections or any further manipulations, except for the occasional removal of the CO_2 absorption at ca. 2300–2400 cm^{-1}. The identification of the polymers was carried out by searching the extensive polymer spectral database of the DCR and spectral assignments (not shown here) were made according to Hummel (2002).

3. Results

3.1. Incidence of debris ingestion

Out of the 24 loggerhead turtles analysed, debris items were found in the gastrointestinal tract of 20 individuals (83%). Anthropogenic items were found in all of the size classes investigated (Fig. 2A), from the smallest (10–21 cm, CCL) to the largest (61–70 cm, CCL) individuals (Table 1). Incidence of debris ingestion was more prominent with increasing turtle size, with all of the individuals > 31.1 cm ($n = 12$) having ingested debris (Fig. 2A). Debris incidence was higher in the intestine (71%), followed by stomach (44%) and oesophagus (9%) (Fig. 2B).

3.2. Quantity of ingested debris

A total of 380 debris items was recovered from all the loggerheads sampled (ranging between 0 and 139 items per turtle and a dry mass of 0 to 7.01 g of debris per turtle). Average number of items was 15.83 ± 6.09 (± SE) per individual, corresponding to a mean dry mass of 1.07 ± 0.41 g. There was a significant difference in the dry mass of debris ingested between size classes (H = 13.06, $p < 0.05$) but not for the number of the debris items (Fig. 3A & B). However, when split into small (≤ 40 cm) and large (> 40 cm) loggerheads, there were no significant differences in the number ($T = 596.5$, $p = 0.199$) and dry mass ($T = 606.5$, $p = 0.135$) of debris ingested. The average number of debris and dry mass in the intestines was significantly higher compared to the oesophagus (H = 9.9, $p < 0.05$ for number; H = 8.12, $p < 0.05$ for dry mass (Fig. 3C & D)).

A serious obstruction of the digestive tract was detected in one individual. The turtle had a metal hook (type: ANCORA 16–17 used by the Portuguese and Spanish pelagic longline fleet) perforating the oesophagus. Though this hook was not considered as a debris item (since it is likely the result of accidental bycatch), its presence caused an accumulation of debris within the oesophagus (Fig. 4A; 4B). For this individual, it is likely that the ingestion of marine debris and subsequent blockage of the oesophagus, restricted feeding and was a principal factor for its death.

3.3. Debris composition

All 380 debris recovered from the loggerheads were plastic, predominantly identified as user plastics (mean: 15.75 ± 6.59 items per turtle), followed by fishing-related plastic (3.00 ± 1.42) and finally by industrial plastic (0.25 ± 0.25) (Fig. 5A). This corresponded to a mean dry mass per turtle of 1.14 ± 0.41 g for user plastics; 0.14 ± 0.11 g for fishing-related plastic and 0.001 ± 0.001 g for industrial plastic (Fig. 5B).

User plastics were principally plastic fragments (67.6%; $n = 213$), followed by the remains of plastic sheets (31.1%; $n = 98$) and foam

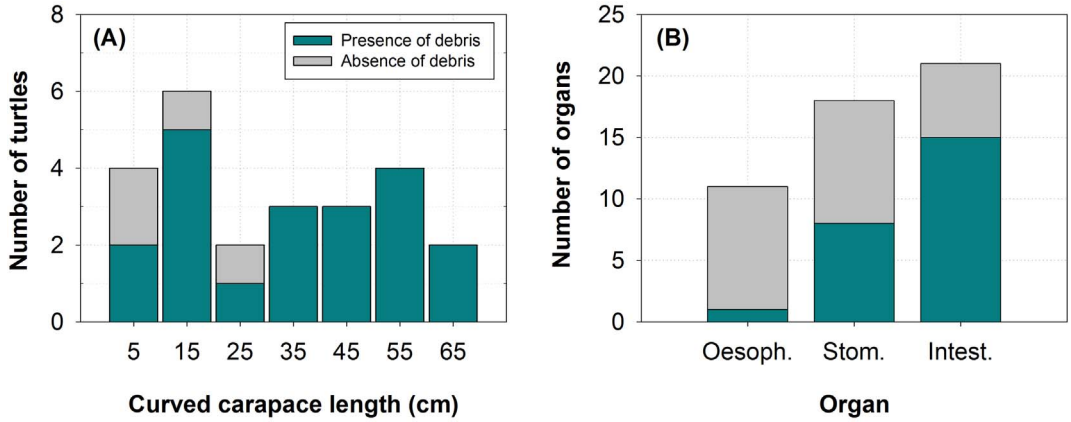

Fig. 2. Occurrence of debris in loggerheads (*Caretta caretta*) (A) of different size classes (CCL) and (B) for different organs.

(1.3%; $n = 4$). Fishing-related plastic was composed primarily of synthetic rope (63%; $n = 38$) and fishing line (37%; $n = 22$). Industrial plastic was represented exclusively by pellets (100%; $n = 5$).

3.4. Maximum length and colour of debris

The maximum length of the debris ranged between 1 mm (plastic fragment) to a maximum of 310 mm (black rope). However, the majority (95%) of the items were smaller than 80 mm (Fig. 6) with an average length of 20.3 ± 1.6 mm (± SE). The mean length of the debris by organ was higher for the oesophagus (94.4 ± 27.3 mm), followed by the stomach (24.9 ± 2.6 mm) and finally by the intestines (16.4 ± 1.6 mm). Overall, there was a positive correlation between the average length of debris items ingested and the size of the turtles (Spearman rho = 0.5; $p < 0.01$) (Fig. 7).

Large microplastics (1–5 mm) represented 25% of the debris recovered from the turtles and were found in 58% of the individuals sampled, mostly in the intestines (94%). Large microplastics were predominantly fragments (87%), followed by small pieces of sheets (8%) and pellets (5%).

For individuals in which large microplastics were encountered, the colour composition of all plastic fragments (large microplastics, *meso* and macroplastics; fragments only) suggests that in certain cases (Fig. 8; e.g. turtle P7549), fragmentation of large items in the gut could have caused the presence of such small fragments. On the other hand, within the same individual, we found the presence of large microplastic fragments of certain colours that were not represented by larger fragments (Fig. 8; e.g. turtle N8431). Throughout all the debris recovered, white was the predominant colour (45%), followed by transparent (21%).

3.5. Plastic identification

The most common polymers identified in loggerheads were polyethylene (PE - 60%), polypropylene (PP - 20%) and different polymer

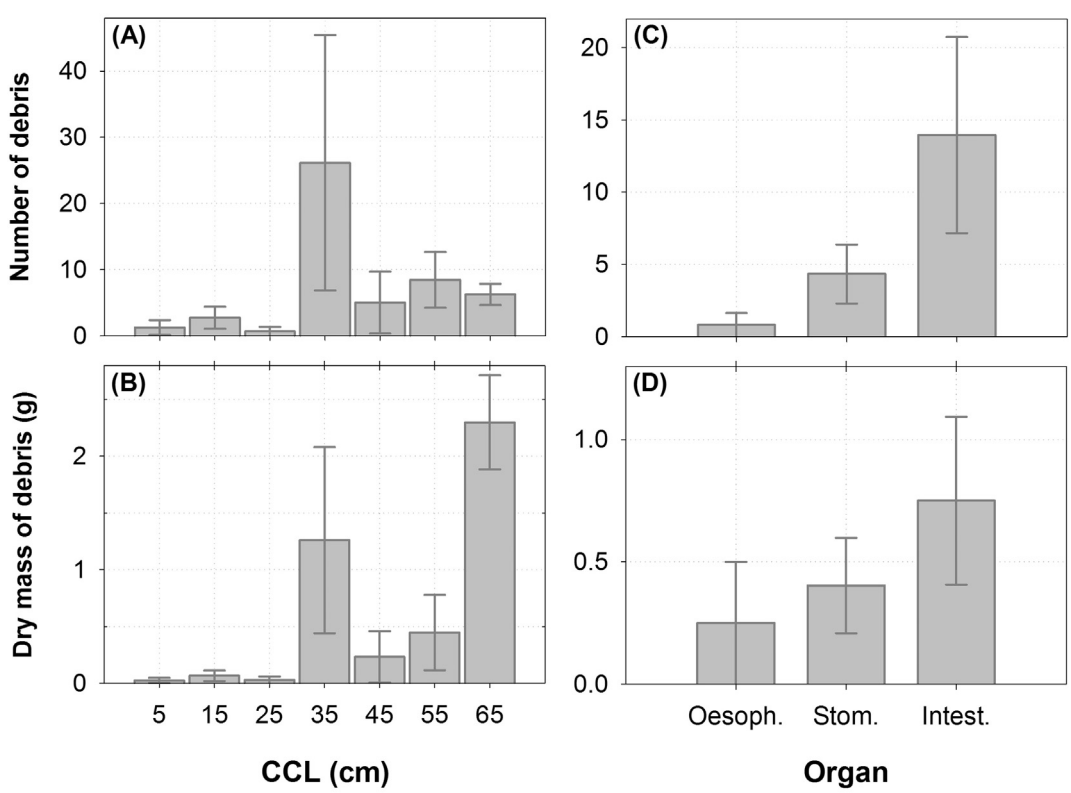

Fig. 3. (A) Average number and (B) dry mass of debris for different size classes (CCL = curved carapace length); (C) average number and (D) average dry mass of debris recovered from different organs of loggerhead turtles (*Caretta caretta*) in the Azores. Error bars indicate standard errors. Oesoph. = oesophagus; Stom. = stomach; Intest. = intestine.

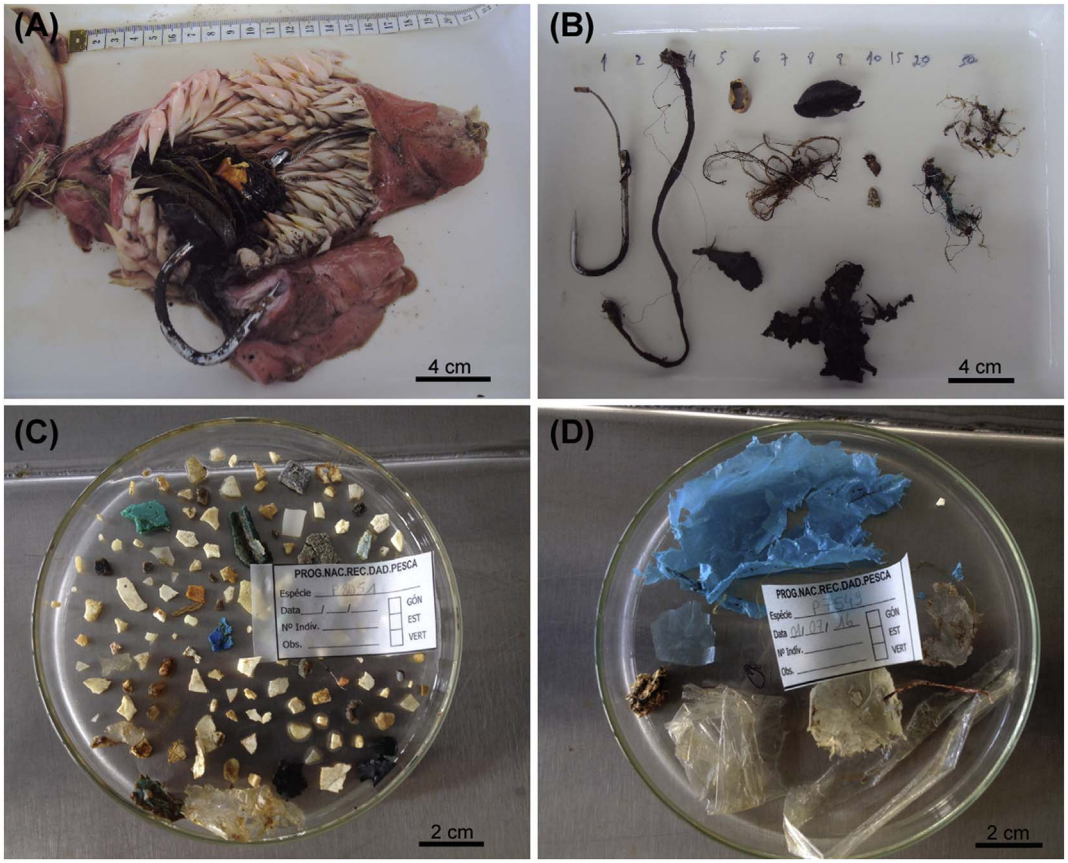

Fig. 4. Marine debris recovered from loggerhead sea turtles (*Caretta caretta*) in the Azores. (A) ANCORA 16–17 hook trapping debris in the oesophagus of a loggerhead (60.5 cm, CCL); (B) debris accumulated around the hook; (C) debris encountered in the intestine of a loggerhead (30.2 cm, CCL) accidentally caught by pelagic longline gear; (D) debris dominated by plastic sheets recovered in the intestine of a loggerhead (42.7 cm, CCL) captured by a pelagic longline.

mixtures (12%). Synthetic polymers identified were PE, PP, copolymer mixtures between PE and PP [PP + P(E:P)], Rayon (synthetic cellulose fibre), Poly(vinyl chloride) (PVC), Poly(vinyl acetate) (PVAc), and Nylon. Two samples were identified as biological samples, whose spectra showed bands that are identified as biological organic compounds (Fig. 9A). In one particular case, the texture and colour of a group of items closely resembled transparent plastic sheets, identified as polyethylene, which were also encountered in abundance within the same stomach (Fig. 9B).

4. Discussion

The current study demonstrates a high occurrence of plastic ingestion in oceanic-stage loggerheads of the North Atlantic. For most of the individuals sampled in this study, ingestion of debris was not identified as the direct cause of death. Disregarding significant evidences of sublethal effects of plastic ingestion (e.g. McCauley and Bjorndal, 1999), debris ingestion is rarely reported as being directly responsible for the death of sea turtles (Casale et al., 2016). Actually, due to their wide digestive tract, loggerheads have the ability to defecate most of the ingested debris (Bugoni et al., 2001; Hoarau et al., 2014). We found only one loggerhead for which the ingestion of debris most likely played an important role in its death. The individual had a hook punctured in the oesophagus, trapping a large amount of plastic debris and severely blocking the oesophagus. This exemplifies how two different independent stressors (in this case fisheries and plastic pollution), can act in combination, leading to cumulative impacts of significant consequences for loggerhead populations.

Overall, we found that 83% of the sampled individuals had ingested debris. However, this estimate is conservative since for some of the individuals we could not sample all of the organs. Analysing the entire digestive tracts of the two incomplete individuals for which we did not encounter any debris, could have increased the incidence of debris ingestion to a maximum of 91%. Such an elevated occurrence of plastic debris in loggerhead turtles was unexpected considering that a previous study looking at diet composition in oceanic-stage loggerhead turtles in the Azores reported the presence of debris in only 25% of the sampled individuals (Frick et al., 2009). Such discrepancy with our results is most likely because the authors were studying the diet, which focuses on the stomach and not the intestines; the portion of the gut where debris are most abundant (this study; Bjorndal et al., 1994; Camedda et al., 2014; Campani et al., 2013; Casale et al., 2016; González Carman et al., 2014; Guebert-Bartholo et al., 2011; Macedo et al., 2011; Nicolau et al., 2016; Tomás et al., 2002; Tourinho et al., 2010). Therefore, examination of the stomach alone ultimately provides an underestimation of debris ingestion, as first suggested by Bjorndal et al. (1994). In addition, the inclusion of fecal samples (obtained over a short time period) by Frick et al. (2009) maybe another important factor explaining the difference with our results. Debris can remain in the gut for at least 41 days before being defecated (Hoarau et al., 2014). Therefore, extended periods of observations in controlled captivity conditions are important prerequisites for obtaining an accurate assessment of debris ingestion by turtles when using fecal analysis.

Comparison with other studies of the incidence of debris ingestion is a major challenge because of differences in the methods, size of the debris items considered, organs sampled, life history stages, size range of the individuals sampled, etc. (Nelms et al., 2016). Keeping in mind such limitations, our findings on the incidence of debris ingestion are in the high end of the studies looking at Atlantic and Mediterranean loggerhead populations (Bugoni et al., 2001 (10%); Tomás et al., 2002 (75.9%); Casale et al., 2008 (48.1%); Lazar and Gračan, 2011 (35.2%); Campani et al., 2013 (71%); Camedda et al., 2014 (14%); Casale et al.,

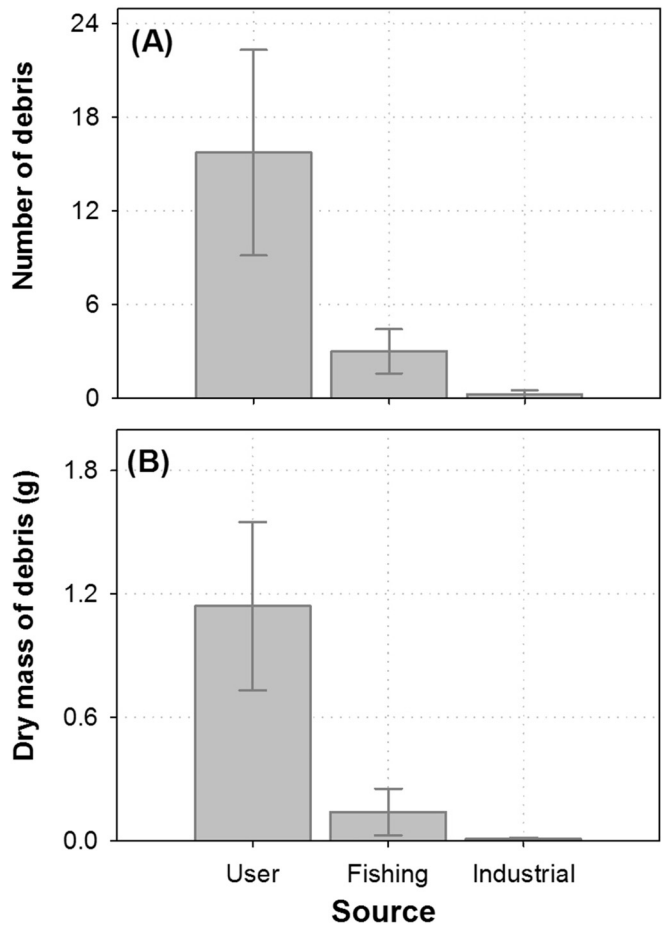

Fig. 5. (A) Average number and (B) dry mass of plastic debris ingested by loggerhead turtles (*Caretta caretta*) grouped by debris source.

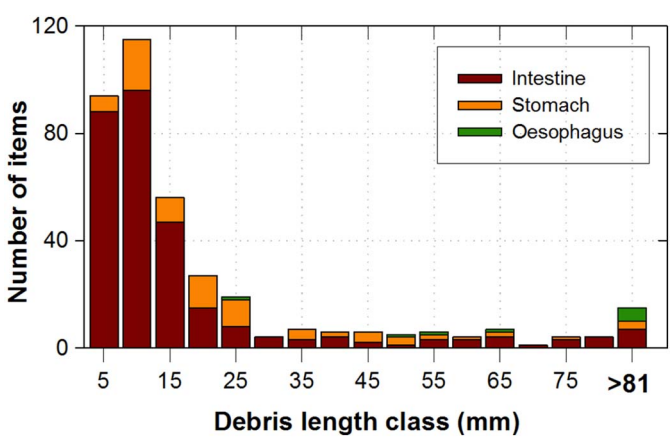

Fig. 6. Size frequency distribution of all the plastic debris recovered from different organs of loggerhead turtles (*Caretta caretta*) in the Azores.

2016 (80%); Nicolau et al., 2016 (59%)). This is likely because our study focused on oceanic-stage loggerheads that forage exclusively in the open ocean and seamounts (Santos et al., 2007). This life-history stage displays characteristics related to their feeding ecology but most importantly, their spatial distribution which increases the likelihood of debris ingestion (Schuyler et al., 2014a). The archipelago is located at the edge of the North Atlantic subtropical gyre, that loggerhead hatchlings use to disperse into the open ocean (Bolten, 2003) and that is known to be an accumulation zone of marine debris (Law et al., 2010). It has often been hypothesised that it is this association that makes them particularly vulnerable to plastic pollution (Schuyler et al., 2015). In

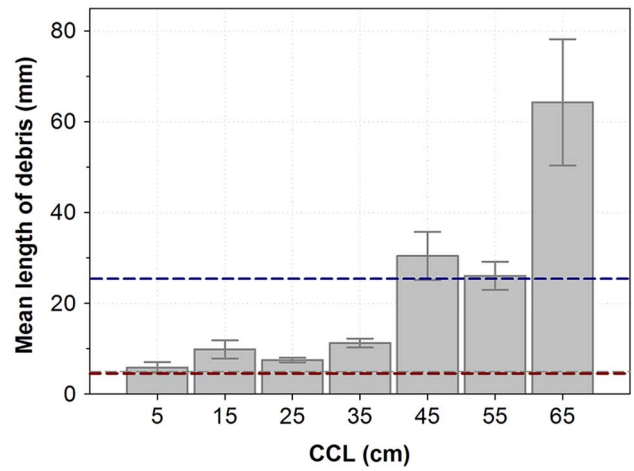

Fig. 7. Mean length of debris items recovered from loggerhead turtles (*Caretta caretta*) of different size classes (CCL: curved carapace length). Red dashed line is positioned at 5 mm (i.e. upper size limit of large microplastics); blue dashed line is positioned at 25 mm (i.e. upper size limit of mesoplastics). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

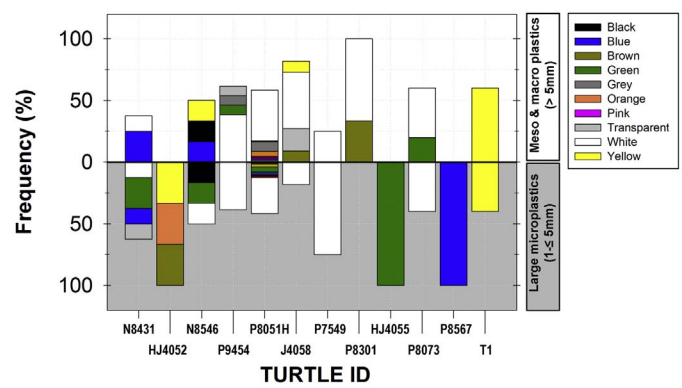

Fig. 8. Frequency of different coloured plastic debris (fragments only) recovered from every loggerhead turtles (*Caretta caretta*) where large microplastic fragments (1–5 mm) had been recovered. The debris are subdivided into two different groups based on their maximum length: large microplastics (1–5 mm) and *meso* and macroplastics (> 5 mm). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

addition, contrary to neritic juveniles and adults that feed primarily on benthic organisms, oceanic juveniles feed on pelagic organisms. In the Azores, loggerheads are opportunistic carnivores that prey upon a variety of oceanic and pelagic organisms (Frick et al., 2009) that can easily be mistaken for plastic (Schuyler et al., 2014b).

Quantities of ingested plastic are difficult to compare with other studies because of the different metrics used (e.g. number vs. dry mass) and because some studies compute average values without including individuals that did not ingest plastic (Kühn et al., 2015). In addition, there is a general inconsistency in the lower size limit of the debris considered by different authors which undeniably influences the estimated quantities ingested by sea turtles. For example, Nicolau et al. (2016) did not include debris smaller than 5 mm, while Tomás et al. (2002) only considered items longer than 10 mm. These authors argued that items of these size classes result from fragmentation of larger items or through incidental ingestion and including them would lead to an overestimation of debris ingestion. However, we found that for some individuals, gut contents only included items ≤ 5 mm. Therefore, disregarding this size class would not only significantly reduce the observed incidence of plastic ingestion but also overlook the ingestion of industrial plastic (such as pellets). Although our assessment was limited to large microplastics (1 to 5 mm), including smaller microplastics (< 1 mm) when monitoring debris ingestion in sea turtles is essential to

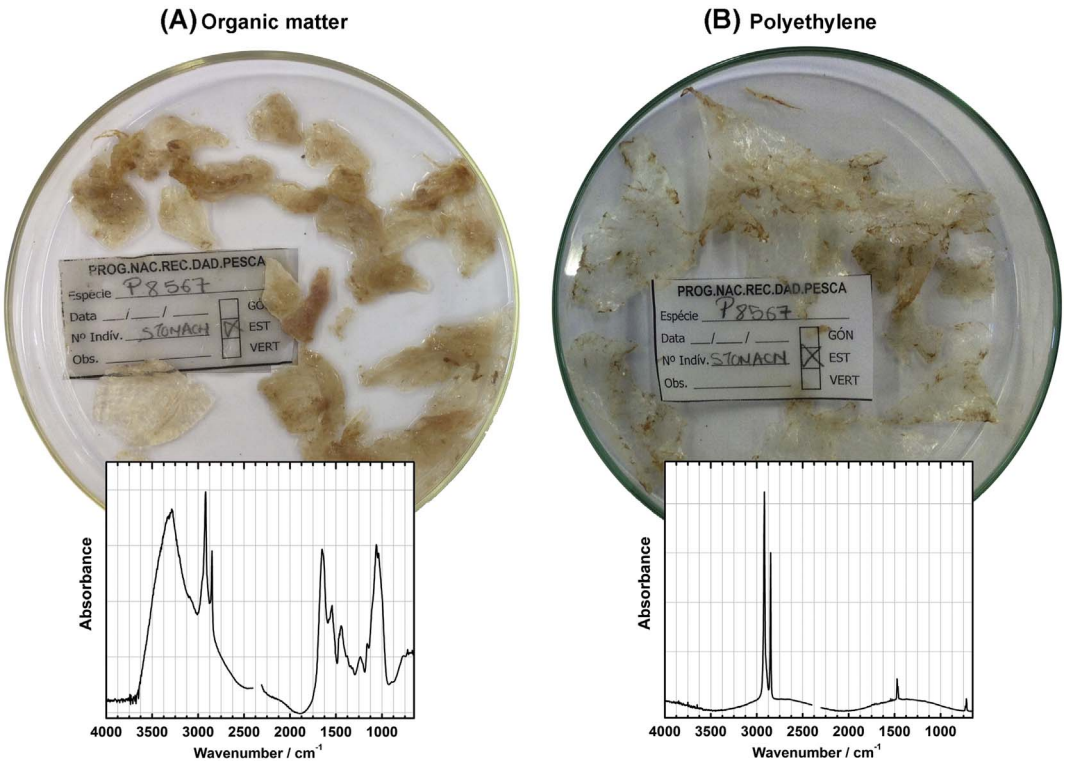

Fig. 9. Similarities in the colour, texture and shape between (A) natural prey and (B) transparent plastic sheets, and respective infrared spectra. Both items were recovered from the stomach of a loggerhead (57.4 cm, CCL) in the Azores. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

understand sublethal effects since the large surface area to volume ratio of microplastics indicate that they adsorb a wide variety of contaminants from the surrounding seawater that can leach into the turtle's tissues upon ingestion (Koelmans, 2015).

White and transparent plastic fragments and sheets were the dominant items found in the loggerheads sampled. This was similar to most studies on debris ingestion in sea turtles (e.g., Camedda et al., 2014; Schuyler et al., 2012; Nicolau et al., 2016). Jellyfish (e.g. *Velella velella* and *Pelagia noctiluca*) are the most important dietary resources for this life history stage in the Atlantic (Frick et al., 2009) and could easily be mistaken for white and transparent plastic debris (Fig. 9). Alternatively, the predominance of white and transparent plastic items in the gut could also reflect the higher abundance of these debris in the region (Pham et al., unpublished data).

Loggerhead turtles have been proposed as a potential candidate indicator species for monitoring indicator 10.2.1 associated to Descriptor 10 (Marine Litter) of the MSFD: “Trends in the amount and composition of litter ingested by marine animals.” The results of this study show that monitoring plastic ingestion by oceanic-stage loggerheads inhabiting the Azores could be used to assess temporal and spatial trends in plastic pollution within the scope of the MSFD. However, more research is required to define methodological standards before sea turtles can be used as an indicator but most importantly to assess the implication of plastic ingestion for the conservation status of these endangered animals.

The present study demonstrates that oceanic-stage loggerheads of the North Atlantic are particularly prone to plastic pollution. Their association with the North Atlantic subtropical gyre, where floating debris accumulate, together with characteristics of their feeding ecology likely increases the incidence of debris ingestion compared to neritic life stages.

This study suggests that the increasing quantity of plastic debris in the North Atlantic pose a significant risk for loggerhead populations that are already under pressure of other anthropogenic threats such as fishing activities (Wallace et al., 2013).

Acknowledgments

The authors would like to acknowledge the funds provided by the Gallifrey Foundation and International Union for the Conservation of Nature (IUCN) through the project Azorlit (Project Number - P01495). The study was co-financed by the Regional Government of Sea Affairs (DRAM) and by the project LIXAZ (ACORES-01-0145-FEDER-00053) through the operational program AÇORES 2020. We further acknowledge the project INDICIT that has received funding from the European Union under the Grant Agreement n°11.0661/2016/748064/SUB/ENV.C2. and project COSTA funded by the Marine Turtle Conservation Fund of the U.S. Fish and Wildlife Service (Agreement number F15AP00577). The authors also acknowledge funds provided by the Fundação para a Ciência e a Tecnologia (FCT) through the strategic project (FCT/UID/MAR/04292/2013) granted to MARE. FV is supported by an individual grant of FCT (SFRH/BPD/110294/2015). Partial funding for this project is also from the Archie Carr Center for Sea Turtle Research through support from the Disney Conservation Fund. We would like to extend our thanks to everyone involved in the collection of stranded sea turtles through the “Rede de Arrojamento de Cetaceos dos Açores (RACA)” coordinated by DRAM. Finally we would like to thank two anonymous reviewers for their valuable and insightful comments that improved the manuscript.

References

Bergmann, M., Gutow, L., Klages, M. (Eds.), 2015. Marine Anthropogenic Litter. Springer, Heidelberg, Ger..

Bjorndal, K.A., Bolten, A.B., Lagueux, C.J., 1994. Ingestion of marine debris by juvenile sea turtles in coastal Florida habitats. Mar. Pollut. Bull. 28, 154–158.

Bjorndal, K., Bolten, A., Dellinger, T., Delgado, C., Martins, H., 2003. Compensatory growth in oceanic loggerhead sea turtles: response to a stochastic environment. Ecology 84, 1237–1249.

Bolten, A.B., 2003. Active swimmers - passive drifters: the oceanic juvenile stage of loggerheads in the Atlantic system. In: Bolten, A.B., Witherington, B.E. (Eds.), Loggerhead Sea Turtles. Smithsonian Institution Press, Washington, DC, pp. 63–78.

Bolten, A., Bjorndal, K., Martins, H., Dellinger, T., Biscoito, M., Encalada, S., Bowen, B., 1998. Atlantic developmental migrations of loggerhead turtles demonstrated by

mtDNA sequence analysis. Ecol. Appl. 8, 1–7.
Bugoni, L., Krause, L., Petry, M.V., 2001. Marine debris and human impacts on sea turtles in southern Brazil. Mar. Pollut. Bull. 42, 1330–1334.
Camedda, A., Marra, S., Matiddi, M., Massaro, G., Coppa, S., Perilli, A., Ruiu, A., Briguglio, P., de Lucia, G.A., 2014. Interaction between loggerhead sea turtles (*Caretta caretta*) and marine litter in Sardinia (Western Mediterranean Sea). Mar. Environ. Res. 100, 25–32.
Campani, T., Baini, M., Giannetti, M., Cancelli, F., Mancusi, C., Serena, F., Marsili, L., Casini, S., Fossi, M.C., 2013. Presence of plastic debris in loggerhead turtle stranded along the Tuscany coasts of the Pelagos Sanctuary for Mediterranean Marine Mammals (Italy). Mar. Pollut. Bull. 74, 225–230.
Casale, P., Abbate, G., Freggi, D., Conte, N., Oliverio, M., Argano, R., 2008. Foraging ecology of loggerhead sea turtles *Caretta caretta* in the central Mediterranean Sea: evidence for a relaxed life history model. Mar. Ecol. Prog. Ser. 372, 265–276.
Casale, P., Freggi, D., Paduano, V., Oliverio, M., 2016. Biases and best approaches for assessing debris ingestion in sea turtles with a case study in the Mediterranean. Mar. Pollut. Bull. 110 (1), 238–249.
Di Beneditto, A.P.M., Awabdi, D.R., 2014. How marine debris ingestion differs among megafauna species in a tropical coastal area. Mar. Pollut. Bull. 88, 86–90.
van Franeker, J.A., Blaize, C., Danielsen, J., Fairclough, K., Gollan, J., Guse, N., Hansen, P.-L., Heubeck, M., Jensen, J.-K., Le Guillou, G., 2011. Monitoring plastic ingestion by the northern fulmar *Fulmarus glacialis* in the North Sea. Environ. Pollut. 159, 2609–2615.
Frias, J.P.G.L., Otero, V., Sobral, P., 2014. Evidences of microplastics in samples of zooplankton from Portuguese coastal waters. Mar. Environ. Res. 95, 89–95.
Frick, M.G., Williams, K.L., Bolten, A.B., Bjorndal, K.A., Martins, H.R., 2009. Foraging ecology of oceanic-stage loggerhead turtles *Caretta caretta*. Endanger. Species Res. 9, 91–97.
Galgani, F., Hanke, G., Werner, S., Oosterbaan, L., Nilsson, P., Fleet, D., Kinsey, S., Thompson, R.C., van Franeker, J., Vlachogianni, T., Scoullos, M., Veiga, J.M., Palatinus, A., Matiddi, M., Maes, T., Korpinen, S., Budziak, A., Leslie, H., Gago, J., Liebezeit, G., 2013. Guidance on Monitoring of Marine Litter in European Seas. Publications Office of the European Union, Luxembourg.
Galgani, F., Claro, F., Depledge, M., Fossi, C., 2014. Monitoring the impact of litter in large vertebrates in the Mediterranean Sea within the European Marine Strategy Framework Directive (MSFD): constraints, specificities and recommendations. Mar. Environ. Res. 100, 3–9.
Gall, S.C., Thompson, R.C., 2015. The impact of debris on marine life. Mar. Pollut. Bull. 92, 170–179.
González Carman, V., Acha, E.M., Maxwell, S.M., Albareda, D., Campagna, C., Mianzan, H., 2014. Young green turtles, *Chelonia mydas*, exposed to plastic in a frontal area of the SW Atlantic. Mar. Pollut. Bull. 78, 56–62.
Guebert-Bartholo, F.M., Barletta, M., Costa, M.F., Monteiro-Filho, E.L.A., 2011. Using gut contents to assess foraging patterns of juvenile green turtles *Chelonia mydas* in the Paranaguá Estuary, Brazil. Endanger. Species Res. 13, 131–143.
Hoarau, L., Ainley, L., Jean, C., Ciccione, S., 2014. Ingestion and defecation of marine debris by loggerhead sea turtles, *Caretta caretta*, from by-catches in the south-West Indian Ocean. Mar. Pollut. Bull. 84, 90–96.
Hummel, D.O., 2002. Atlas of Plastics Additives. Springer Verlag, Analysis by Spectrometric Methods (ISBN 3-540-42414-8).
Jambeck, J.R., Geyer, R., Wilcox, C., Siegler, T.R., Perryman, M., Andrady, A., Narayan, R., Law, K.L., 2015. Plastic waste inputs from land into the ocean. Science 347, 768–771.
Knowlton, A.R., Hamilton, P.K., Marx, M.K., Pettis, H.M., Kraus, S.D., 2012. Monitoring north Atlantic right whale *Eubalaena glacialis* entanglement rates: a 30 year retrospective. Mar. Ecol. Prog. Ser. 466, 293–302.
Koelmans, A.A., 2015. Modeling the role of microplastics in bioaccumulation of organic chemicals to marine aquatic organisms. A critical review. In: Bergmann, M., Gutow, L., Klages, M. (Eds.), Marine Anthropogenic Litter. Springer, Berlin, pp. 309–324.
Kühn, S., Bravo Rebolledo, E.L., Van Franeker, J.A., 2015. Deleterious effects of litter on marine life. In: Bergmann, M., Gutow, L., Klages, M. (Eds.), Marine Anthropogenic Litter. Springer, Berlin, pp. 75–116.
Law, K.L., Morét-Ferguson, S., Maximenko, N.A., Proskurowski, G., Peacock, E.E., Hafner, J., Reddy, C.M., 2010. Plastic accumulation in the North Atlantic subtropical gyre. Science 329, 1185–1188.
Lazar, B., Gračan, R., 2011. Ingestion of marine debris by loggerhead sea turtles, *Caretta caretta*, in the Adriatic Sea. Mar. Pollut. Bull. 62, 43–47.
Limpus, C.J., De Villiers, D.L., De Villiers, M.A., Limpus, D.J., Read, M., 2001. The loggerhead turtle, *Caretta caretta* in Queensland: feeding ecology in warm temperate waters. Queensland Mus. Mem. 46, 631–645.
Macedo, G.R., Pires, T.T., Rostan, G., Goldberg, D.W., Leal, D.C., Neto, A.F.G., Franke, C.R., 2011. Anthropogenic debris ingestion by sea turtles in the northern coast of Bahia, Brazil. Cienc. Rural 41, 1938–1943.
McCauley, S.J., Bjorndal, K.A., 1999. Conservation implications of dietary dilution from debris ingestion: sublethal effects in post-hatchling loggerhead sea turtles. Conserv. Biol. 13, 925–929.
Morato, T., Machete, M., Kitchingman, A., Tempera, F., Lai, S., Menezes, G., Pitcher, T.J., Santos, R.S., 2008. Abundance and distribution of seamounts in the Azores. Mar. Ecol. Prog. Ser. 357, 17–21.
Morato, T., Kvile, K.Ø., Taranto, G.H., Tempera, F., Narayanaswamy, B.E., Hebbeln, D., Menezes, G.M., Wienberg, C., Santos, R.S., Pitcher, T.J., 2013. Seamount physiography and biology in the north-east Atlantic and Mediterranean Sea. Biogeosciences 10, 3039–3054.
Nelms, S.E., Duncan, E.M., Broderick, A.C., Galloway, T.S., Godfrey, M.H., Hamann, M., Lindeque, P.K., Godley, B.J., 2016. Plastic and marine turtles: a review and call for research. ICES J. Mar. Sci. 73 (2), 165–181.
Newman, S., Watkins, E., Farmer, A., ten Brink, P., Schweitzer, J.-P., 2015. The economics of marine litter. In: Bergmann, M., Gutow, L., Klages, M. (Eds.), Marine Anthropogenic Litter. Springer, Berlin, pp. 367–394.
Nicolau, L., Marçalo, A., Ferreira, M., Sá, S., Vingada, J., Eira, C., 2016. Ingestion of marine litter by loggerhead sea turtles, *Caretta caretta*, in Portuguese continental Waters. Mar. Pollut. Bull. 103, 179–185.
Parker, D.M., Cooke, W.J., Balazs, G.H., 2005. Diet of oceanic loggerhead sea turtles (*Caretta caretta*) in the central North Pacific. Fish. Bull. 103, 142–152.
Perán, A.D., Pham, C.K., Amorim, P., Cardigos, F., Tempera, F., Morato, T., 2016. Seafloor characteristics in the Azores region (North Atlantic). Front. Mar. Sci. 3, 204. http://dx.doi.org/10.3389/fmars.2016.00204.
Richards, Z.T., Beger, M., 2011. A quantification of the standing stock of macro-debris in Majuro lagoon and its effect on hard coral communities. Mar. Pollut. Bull. 62, 1693–1701.
Santos, M.A., Bolten, A.B., Martins, H.R., Riewald, B., Bjorndal, K.A., 2007. Air-Breathing Visitors to Seamounts: sea turtles. In: Pitcher, T.J., Morato, T., Hart, P.J.B., Clark, M., Haggan, N., Santos, R.S. (Eds.), Seamounts: Ecology, Fisheries and Conservation. Blackwell Publishing, pp. 239–244.
Schuyler, Q., Hardesty, B.D., Wilcox, C., Townsend, K., 2012. To eat or not to eat? Debris selectivity by marine turtles. PLoS One 7.
Schuyler, Q., Hardesty, B.D., Wilcox, C., Townsend, K., 2014a. Global analysis of anthropogenic debris ingestion by sea turtles. Conserv. Biol. 28, 129–139.
Schuyler, Q.A., Wilcox, C., Townsend, K., Hardesty, B.D., Marshall, N.J., 2014b. Mistaken identity? Visual similarities of marine debris to natural prey items of sea turtles. BMC Ecol. 14.
Schuyler, Q.A., Wilcox, C., Townsend, K.A., Wedemeyer-Strombel, K.R., Balazs, G., Sebille, E., Hardesty, B.D., 2015. Risk analysis reveals global hotspots for marine debris ingestion by sea turtles. Glob. Chang. Biol. 22 (2), 567–576.
Tomás, J., Guitart, R., Mateo, R., Raga, J.A., 2002. Marine debris ingestion in loggerhead sea turtles, *Caretta caretta* from the Western Mediterranean. Mar. Pollut. Bull. 44, 211–216.
Tourinho, P.S., do Sul, J.A.I., Fillmann, G., 2010. Is marine debris ingestion still a problem for the coastal marine biota of southern Brazil? Mar. Pollut. Bull. 60, 396–401.
Wallace, B.P., Kot, C.Y., DiMatteo, A.D., Lee, T., Crowder, L.B., Lewison, R.L., 2013. Impacts of fisheries bycatch on marine turtle populations worldwide: toward conservation and research priorities. Ecosphere 4 (3), 40.