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Presence of plastic debris in loggerhead turtle stranded along the Tuscany coasts of the Pelagos Sanctuary for Mediterranean Marine Mammals (Italy)

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ABSTRACT

This work evaluated the presence and the frequency of occurrence of marine litter in the gastrointestinal tract of 31 *Caretta caretta* found stranded or accidentally bycaught in the North Tyrrhenian Sea. Marine debris were present in 71% of specimens and were subdivided in different categories according to Fulmar Protocol (OSPAR 2008). The main type of marine debris found was user plastic, with the main occurrence of sheetlike user plastic. The small juveniles showed a mean ± SD of marine debris items of 19.00 ± 23.84, while the adult specimens showed higher values of marine litter if compared with the juveniles (26.87 ± 35.85). The occurrence of marine debris observed in this work confirms the high impact of marine debris in the Mediterranean Sea in respect to other seas and oceans, and highlights the importance of *Caretta caretta* as good indicator for marine litter in the Marine Strategy Framework Directive (MSFD) of European Union.

1. Introduction

Plastic marine debris accumulation and dispersal is a growing problem on a global scale, affecting all marine environments (Moore, 2008; Gregory, 2009). Marine debris, defined as any manufactured or processed solid waste imported into the marine environment (Coe and Rogers, 1997), are proven to have a widespread negative impact on marine wildlife. Although there are various types of debris, plastics (synthetic organic polymers) make up most of the marine litter worldwide. The main sources of marine debris are litter from ships, fishing and recreational boats, and garbage carried into the sea from land-based sources in industrialized and highly populated areas (Derraik, 2002). The threat of marine debris to the marine environment has been ignored for a long time and only in the last decades it has been given serious attention.

Marine organisms may be impacted by litter in various ways. At least 43% of existing cetacean species, all species of marine turtles, approximately 36% of the world's seabird species, and many species of fish have been reported to ingest marine litter (Katsanevakis, 2008).

The entanglement of marine species, especially fish (Sazima et al., 2002), turtles (Carr, 1987), birds (Arnould and Croxall, 1995) and mammals (Shaughnessy, 1980; Beck and Barros, 1991; Arnould and Croxall, 1995) has been frequently described as a serious mortality factor. Ingestion of debris (mainly plastics) in seaturtles, seabirds and marine mammals has often harmful effects, such as a worsening physical condition (Spear et al., 1995), diminished food stimulus (Ryan et al., 1988), blockage of gastric enzyme secretion, lowered steroid hormone levels, delayed ovulation and reproductive failure (Azzarello and Van Vleet, 1987), internal injuries and death following blockage of the intestinal tract (Ryan et al., 1988; Beck and Barros, 1991).

Loggerhead turtles (*Caretta caretta*) are carnivorus, foraging primarily on benthic invertebrates throughout their distribution range. The high diversity in the type of their prey demonstrates versatility in foraging behavior, suggesting that the loggerhead is a generalist (Plotkin and Amos 1990).

On the basis of these considerations the loggerhead turtles can ingest large quantity of plastic debris that can be mistaken for food.

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Another threat of plastic ingestion was that plastics can be a significant carrier of lipophilic chemicals (mainly persistent organic pollutants – POPs) and a source of other pollutants, such as phthalates and bisphenol A, that can potentially affect different organisms inhabiting the sea and the oceans (Teuten et al., 2007).

Chemicals incorporated in, or attracted to plastics floating in seawater like Polychlorinated biphenyls (PCB's) and other contaminants (phthalate and bisphenol A) enter marine food chains (mainly through ingested plastics) with yet unknown, but potentially very negative effects (Ryan et al., 1988; Bjorndal et al., 1994). The physical and chemical effects on sea turtles caused by marine debris are well described in the literature (National Research Council, 1990; Hutchinson and Simmonds, 1991). The debris may not be lethal at low ingestion levels; however, they can cause side effects that may increase the probability of death (Hutchinson and Simmonds, 1991). An example of such side effects is nutrient dilution, which occurs when non-nutritive items displace food in the gut, affecting the nutrient gain and consequently the growth and/or the reproductive output (McCauley and Bjorndal, 1999).

There are few works containing information about solid debris ingestion by sea turtles in the Mediterranean Sea (Gramentz, 1988; Tomás et al., 2002; Casale et al., 2008; Lazar and Gračan, 2011).

C. caretta, the most common marine turtle species present in the Mediterranean basin, is listed as an Endangered species in the International Union for Conservation of Nature (IUCN) Red List of Threatened Species (IUCN, 2010), widespread over the entire Mediterranean basin (Margaritoulis et al., 2003). Although large numbers of Atlantic turtles enter in the Mediterranean (Laurent et al., 1998; Carreras et al., 2006a; Casale et al., 2008), genetic markers indicate that this population is relatively isolated from the Atlantic populations (Laurent et al., 1998). One of the most distinctive characteristics of the Mediterranean loggerhead population is the smaller adult size in comparison with other populations around the world (Dodd, 1988; Hatase et al., 2002; Margaritoulis et al. 2003). This may represent an adaptation to peculiar conditions, such as poor trophic resources or short migrations (Tiwari and Bjorndal, 2000), and be due to earlier sexual maturation, to slower growth, or to both. Considering the very large number of turtles estimated to be captured in fishing gear and the associated mortality (Casale et al., 2008), it is particularly urgent to assess maturation time and to develop reliable population dynamics models that can help to understand the impact of anthropogenic threats occurring in the basin.

In this context the first objective of the present study was to assess the presence and the abundance of marine debris in the gastrointestinal tract of loggerhead turtle stranded along the Tuscany coasts of the Pelagos Sanctuary (Italy).

The second goal of this work was to quantify and characterize the different types of plastic ingested by sea turtle using the OSPAR protocol (Van Franeker et al., 2011) developed for the sea bird *Fulmar glacialis*.

This paper supports the proposals to choose this species as bioindicator for marine litter in the Mediterranean Sea (descriptor 10 of the Marine strategy framework directive), as reported in the Marine litter technical recommendations for the implementation of MSFD requirements (*MSFD GES Technical Subgroup on Marine Litter* 2011; Matiddi et al., 2011).

2. Material and methods

During the period 2010–2011, 31 stranded loggerhead turtles were collected (CITES Nat. IT025IS, Int. CITES IT 007) along the Tuscany coasts by the staff of Biomarkers laboratory Department of Environment, earth and physical sciences, University of Siena in collaboration with the Environmental Protection Agency of Tuscany Region (ARPAT). The animals were stranded or accidentally bycaught into the Pelagos Sanctuary (North Tyrrhenian Sea) and along the Tuscany coast (Fig. 1).

The animals were subject to necropsy to determine the leading cause of death. Each animal was dissected, biometric parameters were taken and the sex of turtles was determined by visual examination of gross gonadal morphology (Wyneken, 2001). The mean size of the 31 specimens of loggerhead turtle was 51.4 ± 12.2 cm CCL (mean ± SD; CCL: curve carapace length), with CCL range of 29.0–73.0 cm. The mean weight was 19.9 ± 13.0 kg, with weight range of 3.0–50.0 kg.

The main organs (liver, kidney, muscle and adipose tissues) were collected for chemical analysis.

The marine litter determination in the digestive tract of loggerhead turtle was carried out according to Van Franeker et al., 2011 (Table 1) developed for the bird *F. glacialis* and adapted to the Mediterranean loggerhead turtle according to protocol recommendations made in publications mentioned before (*MSFD GES Technical Subgroup on Marine Litter* 2011; Matiddi et al., 2011).

The entire gastrointestinal tract was removed and subdivided into 3 parts: oesophagus, stomach and intestine, using particular attention to not mix the contents. These sections were analysed separately. The contents of each one were drained using a sieve of 1 mm mesh size and overall weight were recorded. After, the content were rinsed with cold water in the same sieve to remove food remains and natural debris (e.g. wood particles, pebbles and sand), Once cleaned all remaining items were placed in petridishes for identification and sorting under stereo-microscope using the same categorization as in Fulmar Protocol analysis.

We determined dry mass (d.m.) of debris samples by weighting the debris (±0.01 g); masses < 0.01 g were recorded as BDL (below detection limit).

All data were analysed using Excel (Microsoft) for frequency of occurrence and graph and Statistica 7.0 (Statsoft) for the regression analysis (Pearson correlation test). Ingestion of debris was quantified as the frequency of occurrence. With regard to the litter, loggerheads were arbitrarily split into two groups according to CCL: small juveniles (CCL < 40 cm), which are going through the transitional pelagic-neritic life stage, and neritic individuals (CCL > 40 cm), which predominantly feed on the sea floor (Casale et al., 2008; Lazar et al., 2008).

3. Results

We examined 31 loggerhead turtles 22 animals (71%) of which had ingested marine debris. Marine debris appeared in the

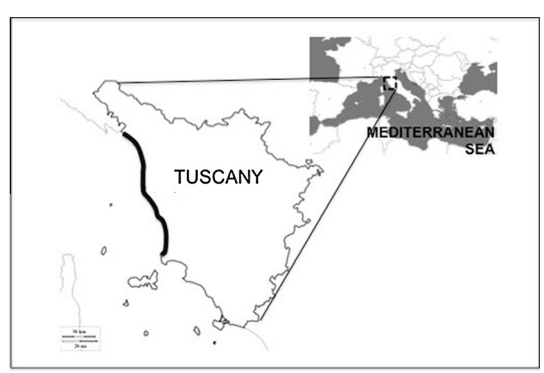

Fig. 1. Sampling area of stranded loggerhead turtle.

Table 1
Categories for characterization of marine debris based on the OSPAR Fulmar Protocol (Van Franeker et al., 2011).

1. Plastics
   1.1.Industrial plastic pellets
   1.2.User plastics
      1.2.1. Sheetlike user plastics
      1.2.2. Threadlike user plastics
      1.2.3. Foamed user plastics
      1.2.4. Fragments
      1.2.5. Other (including e.g. cigarettes filters)
2. Rubbish other than plastic
   2.1. Paper; Incl. multi-layers laminates that are dominated by paper as in tetrapacks, and foils of alu like materials
   2.2. Kitchen food
   2.3. Various rubbish (incl. manufactured wood, paint chips, metal, glass, etc.)
   2.4. Fish hook
3. Pollutants
   3.1. Slag/coal
   3.2. Oil/tar
   3.3. Paraffine/chemical
   3.4. Feather lump (of oil or chemical fouled feathers)

intestine, particularly in the last sections, in higher proportion than in the stomach and oesophagus as reported in Table 2.

In 22 specimens we found 483 pieces of marine litter, with a range from 1 to 143. The mean number and standard deviation of pieces per turtle was 16.5 ± 29.1.

The total mass of debris ingested by the 22 turtles was 62.37 g. The greater quantity of debris was found into the intestine with a mass of 57.82 g, followed by the stomach where we collected 3.55 g of marine litter. In the oesophagus we found only 1 g of marine litter, represented by an hook.

Based on the categorization reported by Van Franeker et al., 2011, the *user plastic* category were the most frequent debris in the stomach and the intestine with a total number of 441 items (Table 3). In term of weight, the stomach contained 3.22 g of *user plastic*: the greater part from the sub-category *sheetlike user plastic* (2.02 g); the intestine, instead, had 54.28 g of *user plastic* prevalently from the sub-categories *fragments user plastic* (31.01 g), and *sheetlike user plastic* (19.32 g). Into the oesophagus we did not find any categories of marine debris except for a single hook that was categorized as *hook*. The category *various rubbish was* found in the stomach and in the intestine with a total of 33 items corresponding to a mass of 3.54 g subdivided in 0.32 g in the stomach and 3.22 g in the intestine (Table 3). The pollutant category was found in the stomach and in the intestine for a total of 6 items: 5 items were present in the intestine with a total weight of 0.32 g while one item was present in the stomach (0.01 g). The Figs. 3 and 4 showed the frequencies of occurrence for each categories and sub categories.

Considering the frequency of occurrence within the total marine litter ingested by *C. caretta*, the main category found was the *user plastic* (Fig. 2) with a frequency 91.7%. As reported in Fig. 3 the main sub categories was the *sheetlike user plastics* (70.4%), followed by the *fragments* subcategory 20.3%, and *threadlike* (4.3%). The other types of litter identified were (Fig. 4): various rubbish (e.g. patch; 91%), paper including tetra pack 2 items (6%).

If the color of the ingested plastic debris is considered 224 of the 483 (46%) items found were colored unidentified (category: *other color*), 104 (22%) were blank plastic debris, 89 items (18%) were white plastic debris and 66 (14%) were black plastic debris.

The highest number of marine debris was found in a female specimen (CCL = 68 cm) that had the 90% of pieces categorized as *sheetlike user plastic* ($n = 143$) for a total mass of 17.36 g; the debris length was between 0.5–16 cm and the masses ranged from < 0.01 g (BDL) to 1.07 g. All the debris were found in the intestine with a mass for the *sheetlike user plastic* of 11.65 g; for *fragments* 2.45 g, *other user plastic* 2.05 g and rubbish 1.21 g.

The small juveniles ($n = 4$;<40 cm CCL; Table 4) showed a mean number and a standard deviation of marine debris of 19.00 ± 23.84, while the adult specimens ($n = 18$;>40 cm CCL; Table 4) showed higher values of marine litter than juvenile (26.87 ± 35.85), although differences were not statistically significant.

The regression analysis performed to determine the relationships between the morphometrical parameter of the loggerhead turtles and the numbers and weight of plastic showed a significant correlation between: number of plastic and CCL ($r = 0.46$; $p < 0.05$), CCL and plastic weight ($r = 0.69$; $p < 0.05$), animal weight and plastic weight ($r = 0.64$; $p < 0.05$).

Specimens ($n = 3$) with a high quantity of marine debris did not show the presence of food remains in the gastrointestinal tract.

4. Discussion

This study enabled to check the presence of litter and in particular plastic debris into the gastrointestinal tract of the loggerhead turtle stranded in the north Tyrrhenian Sea (Pelagos Sanctuary). We found a frequency of marine debris that can be considered high if compared to other literature studies conducted on specimens of *C. caretta* in the Mediterranean Sea and Pacific and Atlantic Oceans (Table 5). Only the work of Tomás et al. (2002) conducted on 54 juveniles loggerhead turtle illegally captured by fishermen in Spanish Mediterranean waters shows higher frequencies of plastic debris.

The loggerhead turtles, demonstrate great resistance to debris ingestion in accordance with the apparent low mortality reported in the literature (Tomás et al., 2002). This fact, and the presence of plastic found principally in the last sections of intestines, indicates that probably most of the plastics pass through the gastrointestinal tract of the *C. caretta* and are excreted (Valente et al., 2008). An important future development will be to investigate the effect of the passage of plastics in the gastrointestinal tract, because the plastic may directly enhance the transport and bioavailability of persistent, bioaccumulative and toxic substances. Moreover, contaminants such as phthalates, bisphenol A and polycyclic aromatic hydrocarbons (PAHs) are among the principal constituents of plastics.

Based on the present study, plastic, in particular sheetlike plastic, is the most reported debris in marine turtles. This might be explained by the ubiquity of sheetlike plastic floating debris in the marine ecosystems or by the higher attraction of *C. caretta* for this debris type (Casale et al., 2008). Our results show that the loggerhead turtles are more attracted to the sheet plastic,

Table 2
Mass and number of item of marine debris in C. caretta in different section of Gastrointestinal tract.

	Oesophagus		Stomach		Intestine	
	Weight (g)	No. items	Weight (g)	No. items	Weight (g)	No. items
Sum Mean ± SD	1.11 (1.77%) 0.55 ± 0.77	3 (0.62%) 1.5 ± 0.71	3.55 (5.68%) 0.44 ± 0.31	41 (8.49%) 5.25 ± 5.34	57.87 (92.55%) 1.87 ± 3.83	439 (90.89%) 14.39 ± 28.14

Table 3
Mass and number of item of marine debris in *C. caretta* based on the categorization reported by Van Franeker et al., 2011 in different section of Gastrointestinal tract.

		User plastic								*Varius rubbish*						*pollutant*	
		1.2.1. Sheetlike		1.2.2. Threadlike		1.2.3. Foamed		1.2.4. Fragments		1.2.5. Other		2.1. Paper		2.3. Rubbish		2.4. Hook		3.2. Tar	
		Weight (g)	No. items	Weight (g)	No. items	Weight (g)	No. items	Weight (g)	No. items	Weight (g)	No. items	Weight (g)	No. items	Weight (g)	No. items	Weight (g)	No. items	Weight (g)	No. items
Stomach	Sum	2.02	22	0.18	7	0.05	1	1.8	2	1	1			0.32	8			0.01	1
	Mean ± SD	0.505 ± 0.25	5.5 ± 4.43	0.09 ± 0.06	3.5 ± 3.54			0.6 ± 0.35	1 ± 0.00					0.16 ± 0.00	4 ± 4.24				
Intestine	Sum	19.32	286	0.56	13	0.45	8	31.01	88	2.98	12	0.61	3	2.61	24			0.32	5
	Mean ± SD	1.14 ± 2.77	16.82 ± 31.08	0.14 ± 0.18	3.25 ± 2.06	0.09 ± 0.06	1.60 ± 0.55	2.82 ± 3.56	8.00 ± 9.63	0.99 ± 0.96	4.00 ± 2.65	0.31 ± 0.42	1.50 ± 0.71	0.44 ± 0.42	4.00 ± 3.41			0.08 ± 0.08	1.25 ± 0.50
Oesophagus	Sum	0.02	2													1.1	1		
	Mean ± SD	0.01 ± 0.00	1																

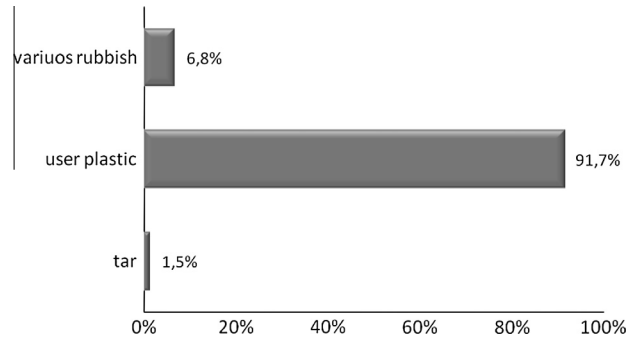

Fig. 2. Frequency of occurrence of different type of marine litter ingested by *C. Caretta* ($N = 31$).

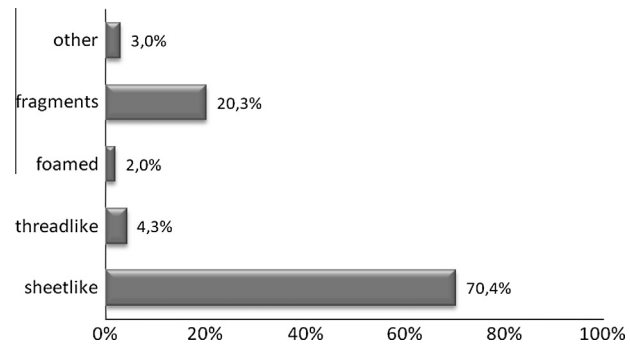

Fig. 3. Frequency of occurrence of the user plastic categories.

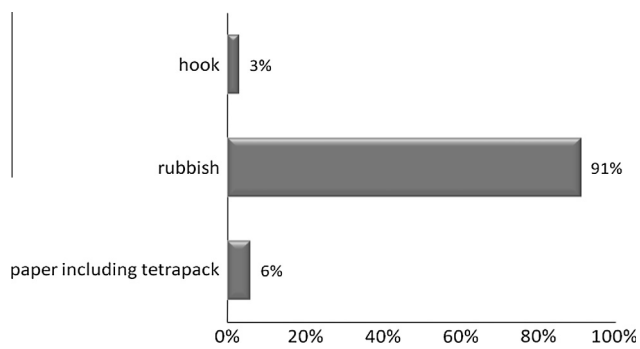

Fig. 4. Frequency of occurrence of rubbish other than plastic.

which they probably mistake for jellyfish, and according to our remarks the loggerhead turtle do not discriminate what they eat by color. Plastic may be disposed of at sea in similar proportions in respect to other types of debris, but it has a high worldwide use, especially by mariners (Tomás et al., 2002). Moreover, due to its lightweight and its environmental persistence, plastic is the most common human debris found in the sea (Laist et al., 1999). In our study we found a statistically significant correlation between the CCL and the weight of plastic ingested by these specimens. To our knowledge in literature this correlation is not found; some authors found evidence of decreased ingestion of plastic with age (Balazs, 1985; Plotkin and Amos, 1990), while other studies have not observed a correlation between CCL and the number of pieces ingested (Casale et al., 2008; Lazar and Gračan, 2011). The occurrence of marine debris observed in this work and in Tomás et al. (2002) confirms the high impact of marine debris in the Mediterranean Sea when compared with other seas and oceans (Atlantic and Pacific). The results obtained in this research confirm

Table 4
Mass and number of item of marine debris in *C. caretta* in different section of Gastrointestinal tract (GI) (CCL, curve carapax lenght).

	Oesophagus		*Stomach*		*Intestine*	
	No. items	Weight (g)	No. items	Weight (g)	No. items	Weight (g)
<40CCL mean ± SD	0.00 ± 0.00	0.00 ± 0.00	2.00 ± 4.00	0.08 ± 0.17	17.50 ± 24.61	0.86 ± 0.59
>40 CCL mean ± SD	0.17 ± 0.51	0.06 ± 0.24	1.89 ± 4.14	0.18 ± 0.31	20.89 ± 33.67	3.02 ± 4.73

Table 5
Frequency of occurrence of marine debris in *C. caretta* in different studies. (CCL, curve carapax length, *N*, number of individuals included in the study).

Study area	References	*N*	CCL range (cm)	Occurrence (%)
Mediterranean Sea				
Tyrrhenian sea (Tuscany coast)	Present study	31	29.0–73.0	71.0
Adriatic sea (Croatia, Slovenia)	Lazar and Gračan (2011)	54	25.0–79.2	35.2
Central Mediterranean (Italy)	Casale et al. (2008)	79	25.0–80.3	48.1
Western Mediterranean (Spain)	Tomás et al. (2002)	54	34.0–69.0	75.9
Central Mediterranean (Malta)	Gramentz (1988)	99	20.0–69.5	20.2
Atlantic ocean				
North–eastern Atlantic (Azores, Portugal)	Frick et al. (2009)	12	9.3–56.0	25.0
North–western Atlantic (Georgia, USA)	Frick et al. (2001)	12	59.4–77.0	0
South–western Atlantic (Brazil)	Bugoni et al. (2001)	10	63.0–97.0	10.0
North–western Atlantic (Florida, USA)	Witherington (1994)	50	4.03–5.63[a]	32.0
Gulf of Mexico (Texas, USA)	Plotkin et al. (1993)	82	51.0–105.0	51.2
Gulf of Mexico (Texas, USA)	Plotkin and Amos (1988)	66	Hatchlings-109.0	47.0
Pacific ocean				
South–western Pacific (Australia)	Boyle and Limpus (2008)	7	4.6–10.6	57.1
Central north Pacific (Hawaii, USA)	Parker et al. (2005)	52	13.5–74.0	34.6

[a] Straight carapace length.

the use of *C. caretta* as a suitable biondicator to measure trends in marine litter specially to monitoring the efficient of the mitigation measures in the Marine strategy framework directive.

Future developments of the research in this field should include the extension of the monitoring network to other Mediterranean areas with the involvement of other research institution. A specific protocol for *C. caretta* within the MSFD will be implemented in collaboration with ISPRA, the Stazione Zoologica Anton Dohrn of Naples, University of Padua, IAMC-CNR Oristano and Environmental Protection Agency of Tuscany Region.

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