



## Ingestion of marine debris by loggerhead sea turtles, *Caretta caretta*, in the Adriatic Sea

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### ABSTRACT

We examined the occurrence of marine debris in the gastrointestinal tract of 54 loggerhead sea turtles (*Caretta caretta*) found stranded or incidentally captured dead by fisheries in the Adriatic Sea, with a curved carapace length of 25.0–79.2 cm. Marine debris was present in 35.2% of turtles and included soft plastic, ropes, Styrofoam and monofilament lines found in 68.4%, 42.1%, 15.8% and 5.3% of loggerheads that have ingested debris, respectively. The dry mass of debris per turtle was low, ranging from <0.01 to 0.71 g, and the ingestion was not significantly affected by sex or body size (all  $p > 0.05$ ). Marine debris averaged  $2.2 \pm 8.0\%$  of dry mass of gut content, with a maximum of 35% found in a juvenile turtle that most likely died due to debris ingestion. Considering the relatively high occurrence of debris intake and possible sub-lethal effects of even small quantities of marine debris, this can be an additional factor of concern for loggerheads in the Adriatic Sea.

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### 1. Introduction

Marine debris, defined as any manufactured or processed solid waste imported into the marine environment (Coe and Rogers, 1997), is proven to have a widespread negative impact on marine wildlife, including physiology-level effects and reduced fitness (Spear et al., 1995; McCauley and Bjorndal, 1999), reproductive failure (Azzarello and Van Vleet, 1987), changes in community structure (Goldberg, 1994; Barnes, 2002; Katsanevakis et al., 2007) to direct death following blockage of intestinal tract or entanglement (review by Derraik (2002)). The number of species affected counts in hundreds and include marine mammals, sea-birds, sea turtles, fish and benthic biota (Laist, 1987). Hence, marine debris represents a major threat to marine life, on top of other anthropogenic stressors affecting marine biodiversity, such as overexploitation and harvesting, chemical pollution, alien species and global climate change (Halpern et al., 2008).

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). In terms of pollution, the Adriatic Sea (Fig. 1) represents one of the World's hot-spots (Halpern et al., 2008). It is a small (surface area: 138,600 km<sup>2</sup>), temperate, shallow, semi-enclosed sea connected to the Mediterranean by the 70 km wide

Straits of Otranto (Cushman-Roisin et al., 2001). The Adriatic coasts, particularly the northern part, are highly populated, with a resident population of about 4 million inhabitants and an additional 18 million tourists during the summer season, resulting in a heavy input of land-based pollutants into the sea (Guerzoni et al., 1984; Marchetti et al., 1989; Picer, 2000). The qualitative and quantitative analyses of chemical pollutants in the Adriatic have been a topic of investigation for more than three decades and have a central role in the Mediterranean Action Plan (UNEP, 1988, 1994). However, to our knowledge no study has addressed the issue of solid waste input into the Adriatic Sea. The only existing information relates to the litter on the sea floor, which concentration (items per hectare) is among the highest along European coasts, after the north-western Mediterranean and the Celtic Sea. If only plastic debris is considered, the Adriatic Sea bottom represents the most polluted sea floor in Europe with 2.63 items/ha (Galgani et al., 2000).

Evaluation of the impact of marine debris on development, survivorship, health and reproduction of sea turtles is highlighted as one of the global research priorities for these threatened marine reptiles (Hamann et al., 2010). The shallow waters of the northern and central Adriatic Sea (<200 m in depth) host one of the most important neritic feeding grounds for the endangered loggerhead sea turtle (*Caretta caretta* Linnaeus 1758) in the Mediterranean (Margaritoulis et al., 2003; Lazar et al., 2004, 2010; Zbinden et al., 2008), while the southern Adriatic is recognized as an important oceanic developmental habitat for this species (Casale et al., 2007). Life history and foraging strategy characterized by high

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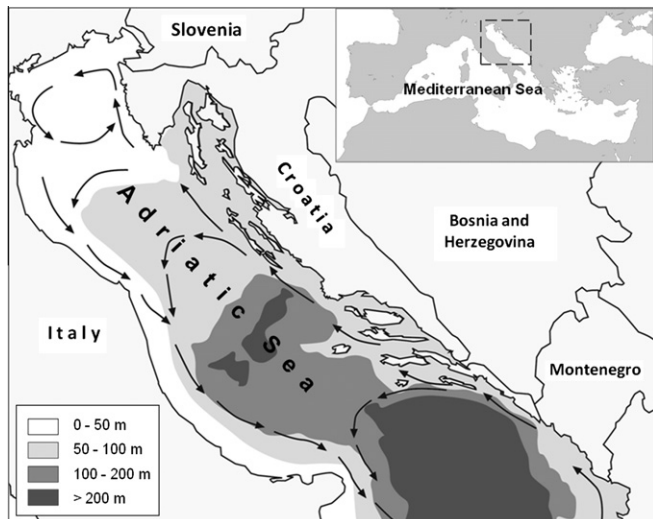


Fig. 1. Adriatic Sea with bathymetry and directions of prevailing sea currents.

ecological plasticity with shifts in diet and habitat use between oceanic and neritic waters (Hawkes et al., 2006; McClellan and Read, 2007; Casale et al., 2008) make loggerheads particularly susceptible to debris ingestion (Lutcavage et al., 1997). Although debris ingestion has been recorded in other large marine vertebrates in the Adriatic (Pribanić et al., 1999; Gomerčić et al., 2006), no such information exists for sea turtles. Therefore, this study investigates for the first time the occurrence of marine debris ingestion by loggerhead sea turtles in the foraging habitats of the Adriatic Sea, and discusses the possible effects on the health status and viability of resident populations.

## 2. Materials and methods

Between June 2001 and November 2004 we collected carcasses of 54 loggerhead sea turtles found dead following incidental capture by fisheries ( $n = 46$ , 85.2%) or dead stranded ( $n = 4$ , 7.4%) in the eastern Adriatic Sea (Slovenia and Croatia; Fig. 1). In the case of four turtles the recovery method was unknown. Most of the animals were found in the northern Adriatic waters ( $n = 49$ ), in the period between May and October ( $n = 50$ ). The mean size of turtles, measured as a notch-tip curved carapace length (CCL; Bolten, 1999) was  $42.7 \pm 11.5$  cm, with CCL range of 25.0–79.2 cm.

We performed a general necropsy, isolated whole digestive tracts (oesophagus, stomach and intestines), then rinsed the gut contents in clear water on a 1 mm mesh sieve, and preserved digestive samples in 4% buffered formaldehyde. The sex of turtles was determined by visual examination of gross gonadal morphology (Wyneken, 2001), for 53 out of the 54 loggerheads sampled. Later, we isolated marine debris from food remains and natural debris (e.g. wood particles, pebbles and sand), and focused our analysis on man-made debris. Debris samples were air-dried for 24 h, while the rest of gut content of each turtle was dried at 95 °C for the same time period. We then determined dry mass (d.m.) by weighing both debris and the rest of gut contents to  $\pm 0.01$  g; masses  $< 0.01$  g were recorded as BDL (below detection limit). We categorized marine debris by type and colour, and measured maximum length of all pieces  $\geq 1$  cm.

Ingestion of debris was quantified as the frequency of occurrence and percent of dry mass of gut content of each animal. We tested size-related changes in mass, size and number of marine debris ingested using the Spearman rank correlation test. For testing the effect of sex on the frequency of debris intake we used Chi-square test ( $\chi^2$ ), with the application of the Yates correction when

necessary. For turtles that had ingested debris, we additionally compared the differences in the amount (mass and percent of mass of gut content), size and number of debris pieces between sexes and sizes of turtles using the Mann–Whitney U-test. For the latter, loggerheads were arbitrary split into two groups according to CCL: small juveniles (CCL  $< 40$  cm) which are going through the transitional oceanic-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

Of the 54 loggerhead turtles examined, 19 (35.2%) had ingested marine debris. We isolated 82 pieces of marine debris in total, represented by four debris types: plastics (mainly remains of plastic bags and wrapping foils), ropes, Styrofoam and monofilament lines (Table 1). In these 19 turtles, the mean number of pieces per turtle was  $4.3 \pm 6.6$  (range: 1–27), with plastic being the most frequent debris, recorded in 13 loggerheads (68.4%). If colour of the ingested debris is considered, 52.6% loggerheads ( $n = 10$ ) contained only white or translucent debris, 31.6% ( $n = 6$ ) ingested debris of other colours (green, brown, red and black), while in three turtles (15.8%) we have found both white/translucent and coloured debris. Seventy-six pieces of debris, found in 14 loggerheads, were  $\geq 1$  cm in length (mean length:  $3.7 \pm 1.8$  cm; range: 1–16 cm). The mean number of these larger debris pieces per turtle was  $5.4 \pm 7.4$  (range: 1–27). The highest number of debris pieces ( $n = 27$ ), between 1.0 and 5.8 cm in length, were recorded in a female loggerhead (CCL = 40.0 cm), but no evidence suggested that debris ingestion caused its death.

Masses of ingested debris were low, ranging from BDL to a maximum of 0.71 g, with the mean d.m. of  $0.08 \pm 0.18$  g. Debris averaged  $2.2 \pm 8.0\%$  of dry mass of gut contents, with a maximum of 35% recorded in a dead stranded female loggerhead of 51.3 cm CCL whose gut contained almost no food remains (total gut content d.m. = 2.03 g). From the stomach and small intestines of this turtle we isolated 15 debris items (remains of plastic bags and plastic foils) between 1.4 and 16.0 cm in length (mean length:  $5.3 \pm 3.8$  cm). Plastics occupied a large portion of stomach's lumen, although total d.m. of marine debris in this turtle was only 0.71 g. We found no external lesions on the carcass or any obvious pathological changes of inner organs, so it is possible that the death of this juvenile loggerhead was due to debris ingestion. In 13 loggerheads debris was found with extremely low masses ( $< 0.01$  g), represented by 1–3 pieces per turtle (mean number of pieces per turtle:  $1.2 \pm 0.6$ ), and did not contribute directly to their deaths. Eight of these 13 turtles ingested debris  $\geq 1$  cm in length (mean number of pieces per turtle:  $1.3 \pm 0.7$ , range: 1–3; mean size of pieces:  $3.1 \pm 2.1$  cm, range: 1.3–6.6 cm).

No significant correlation was found between the size of loggerheads (CCL) and the mass ( $r_s = 0.219$ ,  $p = 0.367$ ), the percentage of mass ( $r_s = 0.216$ ,  $p = 0.375$ ), the number of pieces ( $r_s = 0.042$ ,  $p = 0.864$ ), or the mean size ( $r_s = 0.380$ ,  $p = 0.180$ ) of the debris ingested. Likewise, the sex of the turtles showed no effect on the frequency of occurrence of marine debris ingestion ( $\chi^2 = 2.20$ ,  $df = 1$ ,  $p = 0.138$ ). In turtles that had ingested marine debris, we have

Table 1  
Type, size and occurrence of marine debris ingested by loggerhead sea turtle *C. caretta* ( $n = 19$ ) in Adriatic Sea (N, number of debris pieces).

Marine debris type	N	Size (cm)	Occurrence (%)
Soft plastics	70	<1–16.0	68.4
Ropes	8	1.5–6.1	42.1
Styrofoam	3	<1–3.2	15.8
Monofilament lines	1	<1	5.3

found no significant differences in the amount (mass and percent of mass of gut content), mean size and the number of debris pieces between sexes and sizes (Mann–Whitney U range = 11.00–32.50, all  $p > 0.05$ ).

#### 4. Discussion

Although marine debris was found in about one third of loggerheads from Adriatic, its occurrence is lower than recently recorded from western and central Mediterranean (75.9% and 48.1%, respectively), but similar to the occurrence of anthropogenic debris in this species in central north Pacific (34.6%; Table 2 and references therein) and in leatherback turtles (*Dermochelys coriacea*) worldwide (Mrosovsky et al., 2009). Similar to leatherbacks, most marine debris consumed by loggerheads belongs to the floating debris such as floating plastics (Plotkin et al., 1993; Casale et al., 2008), which incidence of ingestion showed rapid increase from the late 1960s to the 1980s with levelling off after that (Mrosovsky et al., 2009). Soft plastic was the major debris type also in our study. This might be explained by ubiquitous of soft floating debris in the marine ecosystems or by the higher attraction of loggerheads for this debris type (Casale et al., 2008). In the northern Adriatic Sea, loggerheads exhibit an early ontogenetic habitat shift, so that turtles  $\geq 25$  cm CCL already start to feed upon benthic prey (Lazar et al., 2008). Although the sea floor of Adriatic is highly polluted by marine debris, plastics in particular (Galgani et al., 2000), the occurrence of debris in loggerheads in our study is lower than reported from central Mediterranean where the species exhibits similar early transition to neritic habitats (Casale et al., 2008). Though the differences in marine debris pollution between these two neritic foraging habitats should not be ignored, this may support the assumption that loggerheads are attracted to floating debris more than by debris on the sea floor (Casale et al., 2008). Furthermore, the colour of ingested debris in our study confirms relatively low feeding discrimination by loggerheads, as suggested by Tomás et al. (2002).

Due to their highly opportunistic foraging strategy, loggerheads are more prone to ingest debris (Stanley et al., 1988; Witzell and Teas, 1994; Lutcavage et al., 1997), but the quantity of ingested debris is generally low, expressed either as absolute mass/volume, or as a mass/volume percentage of the diet content (e.g. Bjørndal et al., 1994; Bugoni et al., 2001; Plotkin et al., 1993; Casale et al., 2008). However, Bjørndal et al. (1994) showed that ingestion of even small quantities of marine debris, measured in a few grams (e.g. 2.2 and 3.2 g) or in small percentages (4.6% and 5.8%, respectively) can have lethal consequences for animals due to gut obstruction. Similarly, the death of four sea turtles from Brazil

was attributed to blockage of the digestive tract by 1.4–3.2 g of anthropogenic debris (Bugoni et al., 2001). In case of one of our juvenile loggerheads (CCL = 51.3 cm), 15 pieces of ingested soft plastic weighted only 0.71 g, but this was sufficient to occupy the major part of stomach and to probably cause the death of this individual. On the other hand, debris ingestion was not the death cause in a smaller juvenile (CCL = 40.0 cm) which contained the highest number of debris pieces recorded in our study ( $n = 27$ ). This supports a finding of Bjørndal et al. (1994), who suggested that a given amount of debris can pass throughout the gut of a turtle many times without becoming lodged, but during one transit, debris can become orientated in such way as to block the gut and result in the death of the animal. Moreover, beside direct death, debris ingestion also operates on sub-lethal levels, causing dietary dilution and reduced energy intake, such as observed in posthatchlings (McCauley and Bjørndal, 1999). Therefore, possible sub-lethal effects of debris ingestion even in 13 turtles where the mass of ingested debris was  $< 0.01$  g cannot be fully excluded.

In case of loggerheads from our study, debris ingestion (mass, mass percentage, number of pieces and the mean size of ingested debris) was not significantly affected by age (body size), or sex of turtles (frequency of marine debris ingestion). These results can be explained by the life history of the species (Casale et al., 2008) and the accessibility of the same food resources (both in neritic foraging habitats and throughout the water column) to all turtles included in this study, which makes them evenly susceptible to debris ingestion no matter of the size or sex. Similar results for the anthropogenic debris in regard to correlation with the size of turtles were also found by Bjørndal et al. (1994), Bugoni et al. (2001) and Tomás et al. (2002), although some authors found evidence of decreased ingestion of plastics with age (Plotkin and Amos, 1990; Balazs, 1985).

Despite high concentration of marine debris on the sea floor (Galgani et al., 2000), the frequency of debris ingestion by loggerheads in neritic foraging areas of the Adriatic Sea is on the lower end in comparison to other populations of this species elsewhere (Table 2). No information on the floating debris loads exists for Adriatic so far, but ingestion of this type of debris was recorded not only by our study, but also in other endangered species of large marine vertebrates, such as striped dolphin (Pribanić et al., 1999) and Cuvier's beaked whale, and in case of the latter with the fatal consequences (Gomerčić et al., 2006). Out of 54 loggerheads which we investigated for the presence of marine debris in their gastrointestinal tract, only one turtle (1.9%) might died directly because of debris ingestion. However, on top of incidental catch (Lazar and Tvrtković, 1995; Casale et al., 2004), the presence of marine debris in about one third of loggerheads in Adriatic foraging habitat is an

**Table 2**

Frequency of occurrence of marine debris ingestion in loggerhead sea turtles, *C. caretta* (CCL, curved carapace length; N, number of individuals included in the study).

Locality	N	CCL range (cm)	Occurrence (%)	References
<i>Mediterranean Sea</i>				
Adriatic Sea (Croatia, Slovenia)	54	25.0–79.2	35.2	Present study
Central Mediterranean (Italy)	79	25.0–80.3	48.1	Casale et al. (2008)
Western Mediterranean (Spain)	54	34–69	75.9	Tomás et al. (2002)
Central Mediterranean (Malta)	99	20.0–69.5	20.2	Gramentz (1988)
<i>Atlantic Ocean</i>				
North-eastern Atlantic (Azores, Portugal)	12	9.3–56.0	25.0	Frick et al. (2009)
North-western Atlantic (Georgia, USA)	12	59.4–77.0	0	Frick et al. (2001)
South-western Atlantic (Brazil)	10	63–97	10	Bugoni et al. (2001)
North-western Atlantic (Florida, USA)	50	4.03–5.63 <sup>a</sup>	32	Witherington (1994)
Gulf of Mexico (Texas, USA)	82	51.0–105.0	51.2	Plotkin et al. (1993)
Gulf of Mexico (Texas, USA)	66	Hatchlings – 109	47.0	Plotkin and Amos (1988)
<i>Pacific Ocean</i>				
South-western Pacific (Australia)	7	4.6–10.6	57.1	Boyle and Limpus (2008)
Central north Pacific (Hawaii, USA)	52	13.5–74.0	34.6	Parker et al. (2005)

<sup>a</sup> Straight carapace length.

additional matter of concern due to the fact that even small amount of debris can kill a sea turtle and low predictability of such mortality. Hence a reduction of the input of debris, especially plastic, in marine environment and monitoring of temporal patterns in debris ingestion should be considered in conservation planning for large marine vertebrates in the Adriatic Sea.

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