



Contents lists available at ScienceDirect

## Marine Environmental Research

journal homepage: [www.elsevier.com/locate/marenvres](http://www.elsevier.com/locate/marenvres)

## Interaction between loggerhead sea turtles (*Caretta caretta*) and marine litter in Sardinia (Western Mediterranean Sea)

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## ARTICLE INFO

## Article history:

Received 29 September 2013

Received in revised form

6 December 2013

Accepted 9 December 2013

## Keywords:

Sea turtles

Rescue centre

Marine litter

Faecal pellet

Gut content

Plastics

Mediterranean Sea

Marine Strategy Framework Directive

## ABSTRACT

Anthropogenic debris in the environment affects many species that accidentally ingest it. The aim of this study is to evaluate the quantity and composition of marine litter ingested by loggerheads in Sardinia, thus supplying for the lack of data in the existing literature for this area. Seventeen of the 121 (14.04%) monitored turtles presented debris in their digestive tracts. Litter in faecal pellet of alive individuals ( $n = 91$ ) and in gastro-intestinal contents of dead ones ( $n = 30$ ) was categorized, counted and weighed. *User plastic* was the main category of ingested debris with a frequency of occurrence of 13.22% of the total sample, while *sheet* (12.39%) and *fragments* (9.09%) were the most relevant sub-categories. This study highlights for the first time the incidence of litter in alive turtles in Sardinia. This contribution improves the knowledge about marine litter interaction on *Caretta caretta* as bio-indicator. Results will be useful for the Marine Strategy implementation.

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### 1. Introduction

In recent years, many important directives for the protection and restoration of coastal ecosystems have been issued worldwide (Borja et al., 2010). Considering the case of Europe, the European Commission developed the 2008/56/EC Marine Strategy Framework Directive (MSFD; European Commission, 2008) with the aim to achieve a Good Environmental Status (GES), and member states are required to implement an efficient management of marine resources (Borja et al., 2010, 2011, 2013; Cardoso et al., 2010; Claussen et al., 2011; Galgani et al., 2013; Mee et al., 2008; Suarez de Vivero and Rodriguez Mateos, 2012).

“Marine Litter” has been chosen as the descriptor 10 of the MSFD but there are still few data about debris abundance,

distribution and degree of impact on marine species, especially in Mediterranean. For this descriptor, GES is reached when “*properties and quantities of marine litter do not cause harm to the coastal and marine environment*” (Cardoso et al., 2010; European Commission, 2008; Galgani et al., 2010). Most of the waste accumulated in the sea is land-based (Galgani et al., 2013; Law et al., 2010; MEDPOL, 2010), and it inevitably interacts with marine organisms (Derraik, 2002; Galgani et al., 2013; Gregory, 2009; Laist, 1997). Anthropogenic materials can accumulate on the seafloor, in the water column and on the surface (Aliani et al., 2003; Galgani et al., 2000). Floating particles on the sea surface are advected by ocean currents (Martinez et al., 2009) and up to 80% of the waste accumulated on land, shoreline, ocean surface and seabed is plastic (Barnes et al., 2009).

Many species mistakenly ingest debris such as plastic, mono-filament line, rubber, aluminium foil and tar (Bjorndal et al., 1994; Derraik, 2002). Regularly, fishes (Boerger et al., 2010), birds (van Franeker et al., 2011), cetaceans (de Stephanis et al., 2013) and marine turtles (Campani et al., 2013; Carr, 1987; Lazar and Gračan,

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2011; Tourinho et al., 2010) accidentally swallow micro and macro-plastic debris that is often found in their digestive tracts.

To assess plastic impact on biota several bio-indicators like large marine vertebrates should be employed (Fossi et al., 2012). In the Northern sea, for instance, *Fulmarus glacialis* has been chosen to evaluate marine environmental status (van Franeker et al., 2011).

Sea turtles may ingest plastic bags mistaken for jellyfishes (Mrosovsky, 1981; Mrosovsky et al., 2009; Plotkin et al., 1993) when they feed in neritic and pelagic habitats. Plastic fragments and other anthropogenic materials may be directly responsible for the obstruction of digestive tracts (Bugoni et al., 2001; Di Bello et al., 2006) and the death of sea turtles (Bjorndal et al., 1994). Furthermore, long retention times of plastic debris in the intestine may cause the releasing of toxic chemicals (e.g. phthalates, PCBs) that may act as endocrine disruptors and therefore can compromise the fitness of individuals (Teuten et al., 2009).

The loggerhead sea turtle (*Caretta caretta* Linnaeus, 1758) is the most abundant chelonian in Mediterranean (Casale and Margaritoulis, 2010; Margaritoulis et al., 2003), and it is a migratory species (Bentivegna, 2002; Bentivegna et al., 2007; Papi and Luschi, 1996) that usually frequents Sardinian platform habitat especially during the summer season (de Lucia et al., 2011a). This species, which is listed on the Convention on International Trade in Endangered Species (CITES), has been classified worldwide as “endangered” (IUCN, 2013) and considered as a “priority” species according to the Habitat Directive of the European Union.

In the last decade a lot of injured individuals have been rescued by volunteers, associations, marine vigilance bodies and even research institutes in Sardinian coasts (de Lucia et al., 2011b). In order to get a more efficient rescue system, Sardinian Marine Protected Areas (MPAs) and National Parks (NPs) promoted the creation of a Regional Network for the conservation of Marine Turtles and Mammals, supported by the Regional Body (RAS, 2004), according to RAC/SPA (2004). The most frequent causes of injuries for *C. caretta* in Sardinia are constituted by accidental capture (long-lines, gill nets) and entanglement in anthropogenic objects (ghost nets, nylon ropes, plastic cases) (de Lucia et al., 2011a). The Sinis Rescue Centre (CREs) recovers dozens of dead and alive loggerhead turtles every year from Sardinian coasts and some of them present litter ingestion. Monitoring of gastro-intestinal (GI) contents may therefore be useful to understand the sources of impacts and the causes of death in marine organisms (Galgani et al., 2010; van Franeker et al., 2011).

During 2012, an Italian task group (ISPRA, IAMC-CNR Oristano, SZN “Anton Dohrn” Napoli, University of Siena, University of Padova, ARPA Toscana) proposed the loggerhead turtle *C. caretta* as a target-indicator species for the evaluation of ingested macro litter in an experimental protocol specific for the Mediterranean Sea (Galgani et al., 2013; Matiddi et al., 2011).

The categorization of marine litter is derived from the OSPAR protocol (OSPAR, 2008), used for the fulmar monitoring in the Northern Sea (van Franeker and the SNS Fulmar Study Group, 2008; van Franeker et al., 2011). This protocol has been implemented and adapted to the Mediterranean Sea and now includes the monitoring methodology of marine litter in *C. caretta* (Galgani et al., 2013). The loggerhead turtle is adopted worldwide as bio-indicator of environmental conditions as the pollution contamination (Foti et al., 2009; Keller et al., 2006). However, only few data on distribution and density of marine litter in Sardinian sea are currently available, and characteristics and quantity of plastic ingested by *C. caretta* around the island are still unknown. This study aims at providing the missing data, by evaluating the quantity and composition of marine litter ingested by sea turtles. The different types of items observed in the gut contents and in faecal residual of sea turtles were analyzed according to the “Litter in Biota” protocol included in

“Monitoring Guidance for Marine Litter in European Seas” document (Galgani et al., 2013). The present study exhibits data on litter interaction with biota which support the implementation of MSFD for trends in amount and composition of litter ingested by marine animals, and fills the gap in the data regarding *C. caretta* in the Mediterranean region. This study also aims at improving the current knowledge on the ecology and conservation of the loggerhead sea turtle.

## 2. Material and methods

### 2.1. Study area

The present study was conducted in the Sardinian area, located in the Western Mediterranean basin (Fig. 1). MPAs and NPs play a key role as direct observatories of the sea-shore, and their strategic location along the island coastline represents an efficient monitoring system. Since the creation of the Regional Network (RAS, 2004), about 1850 km of coasts are regularly monitored by the competent local institutions.

### 2.2. Loggerhead sea turtles monitoring

From February 2008 to January 2012, 121 loggerhead turtles ranging between 21 and 73 cm of curved carapace length (CCL) with a mean size of  $51.38 \pm 1.13$  cm (mean  $\pm$  SE) were recovered. Eighteen out of the 44 stranded individuals and 73 out of the 77 turtles hand-picked by fishermen and yachtsmen were still alive.

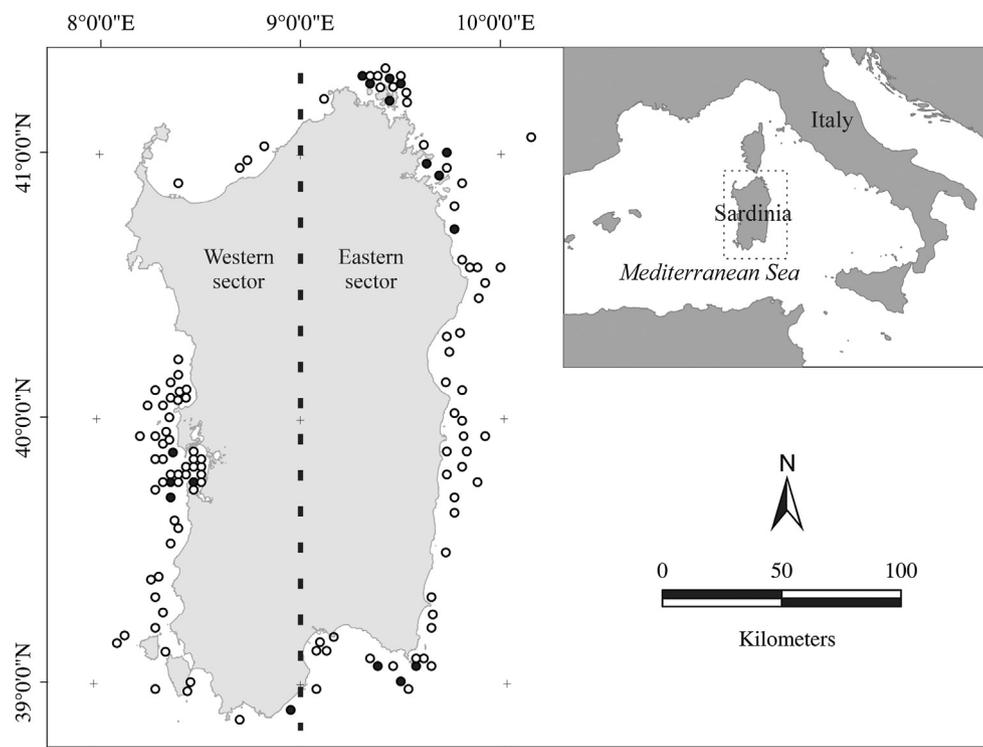
The presence of marine litter was investigated in all the 30 dead animals during necropsies and lab analysis. Digestive tracts were isolated and subdivided in oesophagus, stomach and intestine. The gut contents were rinsed in a 1 mm mesh sieve, avoiding the mixing of ingested materials and food from the different GI tracts. Litter was also monitored in the excreta of 91 alive *C. caretta* held in the rehabilitation tanks since their arrival at CREs, by filtering discharged water into a 1 mm mesh sieve and examining the residual faecal pellet (FP).

Anthropogenic debris was separated from other ingested residual both from GI contents and FP, were preserved in 70% alcohol solution, then dried for 24 h before being sorted and analyzed under the stereo-microscope. The collected items were subdivided in 4 main categories (IND-Industrial plastic, USE-User plastic, RUB-Non plastic rubbish, POL-Pollutants) that include 14 different sub-categories (Table 1), according to the “Litter in Biota” protocol included in “Monitoring Guidance for marine litter in European seas”. The frequency of occurrence (FO) (i.e. percentage of investigated digestive tracts containing litter) was calculated on the entire sample ( $n = 121$ ) both for the total marine litter and for all categories and sub-categories. For each turtle, abundance and weight of every litter category and sub-category were noted (Galgani et al., 2013). Items were subdivided into the 7 observed colours categories (black, blue, green, red, transparent, white, yellow).

Nineteen of the 121 turtles considered in this study interacted with fishing hooks (18 were alive individuals and 1 was a dead one). These data were not included in the analysis because, according to the protocol, fishing hooks on which long-line victims are actively caught are not considered as “marine litter” (Galgani et al., 2013).

### 2.3. Data analysis

Analysis of variance was performed using PRIMER6 software (Plymouth Marine Laboratory, UK) complete of PERMANOVA+ package (Anderson et al., 2008) on Log ( $X + 1$ ) transformed data. Log ( $X + 1$ ) transformed data were tested for homogeneity of dispersion using Permutational Analysis of Multivariate



**Fig. 1.** Study area and distribution map of the recovered sea turtles. Samples distribution within the study area ( $n = 121$ ), divided in Western ( $n = 58$ ) and Eastern Sardinia ( $n = 63$ ). White dots (109) and black dots (17) represent respectively turtles not affected and affected by marine litter.

Dispersions (PERMDISP) (Anderson et al., 2008). Seven litter sub-categories (*probab ind, other, paper, kitchen food, other user, fish-hook, paraf/chem*) were never found in our samples, and for this reason they were excluded from the analysis.

Permanova was ran using three different experimental designs. The first one includes the fixed factor “sub-category” with seven levels (*pellets, sheet, thread, foam, fragments, slag/coal, oil/tar*) and the fixed factor “status” with two levels (alive and dead). This design was implemented in order to estimate whether the number and the weight of items ingested differ among sub-categories, and whether the data from alive and dead individuals are comparable. In order to determine whether the origin of sea turtles affects the composition of ingested litter categories, a second experimental design was implemented using the fixed factor “sector” with two different levels (Western and Eastern Sardinia) and the fixed factor “status” with two levels (alive and dead). The third experimental design includes only the fixed factor “colour”, with seven levels (black, blue, green, red, transparent, white, yellow), in order to evaluate whether the number of items differs among colour categories.

All the analyses were conducted using 9999 random permutations associated with a Monte Carlo test, that permits to have a robust analysis even when the number of unique permutations is low (Anderson et al., 2008). When the  $p$ -values for the factors were significant, pair-wise tests have been performed.

Loggerhead turtles were split into two groups according to CCL: small juveniles (CCL < 40 cm) representing individuals which are going through the transitional pelagic-neritic life stage, and neritic individuals (CCL > 40 cm), which predominantly feed on the seafloor (Casale et al., 2008; Lazar et al., 2010). Regression analysis was performed to test whether there exists a correlation between CCL and number of litter, and between CCL and weight of litter ingested.

### 3. Results

Anthropogenic material collected from ingested matter in alive and dead individuals was made of plastics and pollutants. Seventeen of the 121 (FO = 14.04%) monitored loggerhead turtles presented marine litter in their digestive tracts. Totally 333 items were collected, 276 in alive individuals and 57 in dead ones. Every specimen that interacted with marine litter ingested an average of  $19.58 \pm 10.97$  (mean  $\pm$  SE) items. Eleven of the 91 alive individuals (12.08%) expelled marine litter, while six of the 30 dead turtles (20%) presented litter in their digestive tract (Table 2). In dead turtles, intestine resulted the tract with the highest number of ingested items (40 items; 70.18%), the stomach presented 17 items (29.82%), while no item was found in the oesophagus.

In relation to the loggerhead turtles affected by litter (17 individuals in total, including both dead and alive individuals), USE (*user plastic*) was the main category observed, with an FO of 13.22% ( $n = 121$ ) of the total sample. Within this category, *sheet* was the most frequently ingested sub-category and was found in 15 individuals (FO = 12.39%), followed by *fragments* found in 11 individuals (FO = 9.09%), *thread* found in 5 individuals (FO = 4.13%) and *foam* found in 2 individuals (FO = 1.65%). Occurrence of POL (*pollutants*) category, found in 4 individuals, was estimated as 3.31% represented by *slag/coal* (FO = 1.65%) and *oil/tar* (FO = 1.65%) sub-categories, both of them found in 2 individuals. *Pellets*, sub-category of IND (*industrial plastic*), had the lowest FO (0.82%) and was observed in only one individual (Table 3).

Analysis of categories showed homogeneity in relation of the total abundance, weight and composition among alive and dead turtles, so we considered data from both of them in the analysis (Table 4).

Considering litter sub-categories, the results of Permanova showed significant differences in the number of items per

**Table 1**  
Classification of marine litter ingested by sea turtles. The table is derived from the “Litter in Biota” protocol and includes category and sub-category according to the Monitoring Guidance for marine litter in European seas (Galgani et al., 2013).

| Typology            | Category                         | Sub-category       | Description   |   |
|---------------------|----------------------------------|--------------------|---|---|
| Plastic             | Industrial plastic pellets (IND) | Pellets (ind)      | Industrial plastic granules (usually cylindrical but also oval spherical or cubical shapes exist).  |   |
|                     |                                  | Probab ind (pind)  | Suspected industrial, used for the tiny spheres (glassy, milky...) occasionally encountered.  |   |
|                     | User plastics (USE)              | Sheet (she)        | Remains of sheet, e.g. from bag, cling-foil, agricultural sheets, rubbish bags, etc.  |   |
|                     |                                  | Thread (thr)       | Threadlike materials, e.g. pieces of nylon Ropework, nylon lines e.g. pieces of nylon wire, net-fragments, woven clothing; includes “balls” of compacted such material. |   |
|                     |                                  | Foam (foa)         | All foamed plastic so polystyrene foam, foamed soft rubber (as in mattress filling), PUR used in construction, etc.   |   |
|                     |                                  |                    | Fragments (fra)   | Fragments, broken pieces of thicker type plastics, can be bit flexible, but not like sheetlike materials. |
|                     |                                  |                    | Other (Poth)  | Any other, including elastics, dense rubber, cigarette-filters, balloon-pieces, softairgun bullets.       |
| Non plastic rubbish | Non plastic rubbish (RUB)        | Paper (pap)        | Newspaper, packaging, cardboard, includes multilayered material (e.g. Tetrapack pieces) and aluminium foil.   |   |
|                     |                                  | Kitchen food (kit) | Human food remains (galley wastes) like onion, beans, chickenbones, bacon, seeds of tomatoes, grapes, peppers, melon, etc.  |   |
|                     |                                  | Other user (rva)   | Other consumer waste, like processed wood, pieces of metal, metal air-gun bullets; leadshot, painchips.   |   |
| Pollutants          | Pollutants (POL)                 | Fishhook (hoo)     | Fishing hooks remains (not for hooks on which long-line victims were caught).   |   |
|                     |                                  | Slag/coal (sla)    | Industrial oven slags looks like non-natural pumice) or coal remains.   |   |
|                     |                                  | Oil/tar (tar)      | Lumps of oil or tar (also not $n = 1$ and $g = 0.0001$ g if other particles smeared with tar but cannot be sampled separately).   |   |
|                     |                                  | Paraf/chem (che)   | Lumps or mash of unclear paraffin, was like substances (not stomach oil!) if needed subsample and estimate mass.  |   |

individual (Pseudo- $F = 14.452$ ;  $p = 0.0001$ ). In particular *sheet* (mean number  $11.58 \pm 6.63$  items/ind) showed significantly higher values than the other plastic sub-categories ( $p < 0.05$ ) (Fig. 2a).

The total dry weight of marine litter ingested by alive and dead sea turtles (17 individuals) was 27.76 g with an average of  $1.63 \pm 1.02$  g per individual. The mean weight significantly differed among sub-categories (Pseudo- $F = 4.59$ ;  $p = 0.0005$ ) (Table 4). The most relevant sub-categories were *fragments* ( $1.11 \pm 0.8$  g) and *sheet* ( $0.43 \pm 0.19$  g) as shown in Fig. 2b. Pair-wise test showed that these two sub-categories presented similar weight values ( $p = 0.95$ ), significantly higher than all the other sub-categories ( $p < 0.05$ ). In alive specimen affected by litter ( $n = 11$ ), the main sub-categories were *fragments* (18.52 g) and *sheet* (6.24 g). In dead turtles ( $n = 6$ ) *sheet* represented the main ingested debris (1.15 g), followed by *fragments* (0.45 g).

Litter composition did not differ between the western (5 individuals) and the eastern (12 individuals) sector of Sardinia, both considering the abundance ( $p = 0.89$ ) and the weight ( $p = 0.66$ ) of items (Table 4).

The mean abundance of ingested anthropogenic materials, both in dead and alive sea turtles, significantly differed among colour categories ( $p = 0.0001$ , Table 4). White items were the most abundant ( $7.52 \pm 2.86$ ; 38.43%) and presented significantly higher values than the other colours ( $p < 0.05$ , Table 4). Transparent ( $3.82 \pm 3.08$ ; 19.51%) and black pieces ( $3.47 \pm 1.85$ ; 17.71%) were also quite abundant as shown in Fig. 3.

**Table 2**  
Quantification of marine litter found in the different status of sea turtles. Sample: number of individuals; frequency of occurrence (FO) is expressed in number and in percentage of sea turtles that interacted with litter “number (percentage)”; weight in grams (g) and abundance in number ( $n^\circ$ ) of items are reported.

| Status | Sample | FO          | Abundance ( $n^\circ$ ) | Weight (g) |
|--------|--------|-------------|-------------------------|------------|
| Alive  | 91     | 11 (12.08%) | 276                     | 26.13      |
| Dead   | 30     | 6 (20.00%)  | 57                      | 1.62       |
| Total  | 121    | 17 (14.04%) | 333                     | 27.76      |

Ingestion of marine litter was observed in 5 out of 20 of the smaller individuals (CCL < 40 cm) and in 12 out of 101 of the other ones (CCL > 40 cm). The regression analysis performed to correlate CCL of alive and dead loggerhead sea turtles with abundance ( $r^2 = 0.08$ ;  $p = 0.26$ ) and mass ( $r^2 = 0.19$ ;  $p = 0.08$ ) of ingested litter showed no significant correlation.

#### 4. Discussion

This study highlights for the first time the presence of marine litter in the digestive tracts of sea turtles in Sardinian sea, and in particular it is the first evidence on alive individuals. CReS has given the opportunity to test the “Litter in Biota” Protocol for sampling litter excreted by alive sea turtles (FP analysis) (Galgani et al., 2013) from Sardinian Assessment Area.

Marine litter ingestion may represent a significant threat for *C. caretta* populations that frequent the Mediterranean area (Bjordal et al., 1994); however, during 10 years of activity, we have never found any sea turtle damaged by blockage of the digestive tract.

Satellite telemetry studies indicated that sea turtles are migratory species able to travel long distances, quantified in dozens of

**Table 3**  
Results on categories and sub-categories observed. Frequency of occurrence (FO) in litter categories and sub-categories on dead and alive sea turtles. FP: faecal pellet; GI: gastro-intestinal content; TOT: total number of affected individuals; percentage (%) is referred to the total sample ( $n = 121$ ).

| Category | FO |    |     |      | Sub-category | FO |    |     |       |
|----------|----|----|-----|------|--------------|----|----|-----|-------|
|          | FP | GI | TOT | %    |              | FP | GI | TOT | %     |
| IND      | 1  | 0  | 1   | 0.83 | Pellets      | 1  | 0  | 1   | 0.83  |
|          |    |    |     |      | USE          | 10 | 6  | 16  | 13.22 |
|          |    |    |     |      | Thread       | 3  | 2  | 5   | 4.13  |
|          |    |    |     |      | Foam         | 2  | 0  | 2   | 1.65  |
|          |    |    |     |      | Fragments    | 8  | 3  | 11  | 9.09  |
| POL      | 2  | 2  | 4   | 3.31 | Slag/coal    | 0  | 2  | 2   | 1.65  |
|          |    |    |     |      | Oil/tar      | 2  | 0  | 2   | 1.65  |

**Table 4**

Statistical analysis of litter ingested. Results of Permanova on sub-category, status, colour and sector. df: Degree of freedom; SS: sum of squares; MS: mean square; pseudo-F: value of the pseudo-F statistic; P(MC): *p*-value of Monte Carlo analysis; PERMDISP: *p*-value of the PERMDISP analysis.

| Parameter | Factor                | df  | SS      | MS      | Pseudo-F | P(MC)  | PERMDISP |
|-----------|-----------------------|-----|---------|---------|----------|--------|----------|
| Abundance | Sub-category          | 6   | 27.225  | 4.5376  | 14.452   | 0.0001 |          |
|           | Status                | 1   | 9.1318  | 9.1318  | 0.8654   | 0.8643 |          |
|           | Sub-category × status | 6   | 2.7354  | 0.4559  | 1.4521   | 0.1988 |          |
|           | Residuals             | 98  | 30.769  | 0.31397 |          |        |          |
|           | Total                 | 111 | 60.968  |         |          |        | 0.0001   |
| Weight    | Sub-category          | 6   | 0.68232 | 0.11372 | 4.5926   | 0.0005 |          |
|           | Status                | 1   | 7.5044  | 7.5044  | 3.0307   | 0.0863 |          |
|           | Sub-category × status | 6   | 0.15279 | 2.5465  | 1.0284   | 0.41   |          |
|           | Residuals             | 98  | 2.4266  | 2.4762  |          |        |          |
|           | Total                 | 111 | 3.5322  |         |          |        | 0.0001   |
| Colour    | Colour                | 6   | 20.973  | 3.4956  | 10.214   | 0.0001 |          |
|           | Residuals             | 105 | 35.936  | 0.34225 |          |        |          |
|           | Total                 | 111 | 56.909  |         |          |        | 0.0001   |
| Abundance | Sector                | 1   | 550.1   | 550.1   | 0.23757  | 0.8917 |          |
|           | Status                | 1   | 4076.7  | 1.7606  | 0.1446   | 0.164  |          |
|           | Sector × status       | 1   | 820.81  | 820.81  | 0.35449  | 0.8316 |          |
|           | Residuals             | 12  | 27,786  | 2315.5  |          |        |          |
|           | Total                 | 15  | 32,751  |         |          |        | 0.7419   |
| Weight    | Sector                | 1   | 2565.6  | 2565.6  | 0.68968  | 0.6592 |          |
|           | Status                | 1   | 3655.8  | 3655.8  | 0.98274  | 0.4408 |          |
|           | Sector × status       | 1   | 3127.6  | 3127.6  | 0.84074  | 0.5397 |          |
|           | Residuals             | 12  | 44,641  | 3720.1  |          |        |          |
|           | Total                 | 15  | 53,985  |         |          |        | 0.9185   |

kilometres per day (Bentivegna, 2002; Bentivegna et al., 2007; Luschi et al., 2006; Schofield et al., 2010; Tucker, 2010; Varo-Cruz et al., 2013). We observed that sea turtles in our rescue centre released anthropogenic materials in the faeces for longer than a month of hospitalization, even if most of the litter was expelled within the first 2 weeks. Studies about transit time of substances in gastro-intestinal tracts of loggerhead sea turtles demonstrated that materials (as polyethylene spheres) are expelled in about 10 days (Valente et al., 2008). Therefore, considering the mean distance covered in 10 days by *C. caretta*, the litter defecated during the hospitalization into the tanks is likely to be a sample of debris present around Sardinia (in a range of about 100 nautical miles from coastline). No differences were observed in terms of composition of ingested debris from Eastern and Western coast, showing a similar distribution pattern of litter types in the entire area (Table 4). Certainly, further studies on transit time, particularly of *sheet* plastic, are needed in order to define the spatial-scale resolution in which *C. caretta* is a good bio-indicator. Few studies report data on interaction among sea turtles and litter in Mediterranean (Table 5). In the Gulf of Gabes and Strait of Sicily (Casale et al., 2008) floating plastic debris was found with a high occurrence (FO = 33.7%), in the Adriatic Sea (Lazar and Gračan, 2011) soft plastic were recorded with a high value too (FO = 68.4%) and the maximum plastic incidence (FO = 75.9%) was found in the Western Mediterranean area (Tomás et al., 2002). In the Tyrrhenian Sea litter categorized as *sheetlike* (Campani et al., 2013), which is the analogous to our sub-category *sheet*, showed higher values (FO = 70.4%) than Sardinian results. Several methodologies and different categorizations of marine litter have been used in previous studies making the comparison among data from different areas difficult. A general comparison with the above-mentioned reported studies shows that the observed percentage of interaction with *sheet* plastic litter in Sardinia (FO = 12.39%) is the lowest reported in the Mediterranean (Table 3).

We encourage the application of the MSFD “Guidance on Monitoring of Marine Litter in European Seas” (Galgani et al., 2013) to make more comparable the results coming from different Assessment Areas of Mediterranean regions and sub-regions, and to ensure the beginning of the Marine Litter monitoring activity

within the year 2014. Our study is the first application of the experimental protocol which served for the preparation of the monitoring of the descriptor 10. Results will be useful for the MSFD implementation, to evaluate the Good Environmental Status, to improve the actual knowledge about litter relationships with biota, and to take mitigation measures.

As reported by various authors (Bjorndal, 1997; Frick et al., 2009; Lazar et al., 2010), in the different life stages *C. caretta* feeds on planktonic organisms (e.g. cnidaria or ctenophora) and, with the growth, begins to feed also on benthic preys (e.g. crustaceans, sponges, molluscs). Jellyfishes are more likely to be found, as *sheet* items, in the middle of the water column, and plastic bags are reported to be mistaken for preys by sea turtles (Casale et al., 2008; Mrosovsky et al., 2009; Plotkin et al., 1993).

Loggerhead sea turtles, in particular adult individuals, are able to discriminate colours to find food (Bartol and Musick, 2003), and avoid biting non-preferred preys (Swimmer et al., 2005).

In this study we considered smaller (<40 cm) and sub-adult individuals (>40 cm) and we showed that both of them ingested plastic materials “preyed” on the sea surface and in the water column.

Our results show significant differences in abundance values among items of different colour categories (Table 4). In particular, as shown in Fig. 3, white and transparent *sheet* plastic result the most represented categories, according to Gramentz (1988) and Lazar and Gračan (2011). Those categories seem to be the most widespread colours of floating plastic bags (*sheet* sub-category) around Sardinian coasts (personal observation), which may be the reason of the predominant ingestion’s rate of plastic by sea turtles.

The high occurrence of *sheet* items ingested may be due to their particular suspending pattern that make them more similar to jellyfishes than other plastic objects (*fragments* or *foam*) which positively buoy on the water surface and could be more difficult to come into contact and to approach by sea turtles (Schuyler et al., 2012).

Our results could be explained by the feeding behaviour of *C. caretta* and by the higher presence of light coloured *sheet* materials in the sea water. *Ad hoc* large spatial-scale sampling

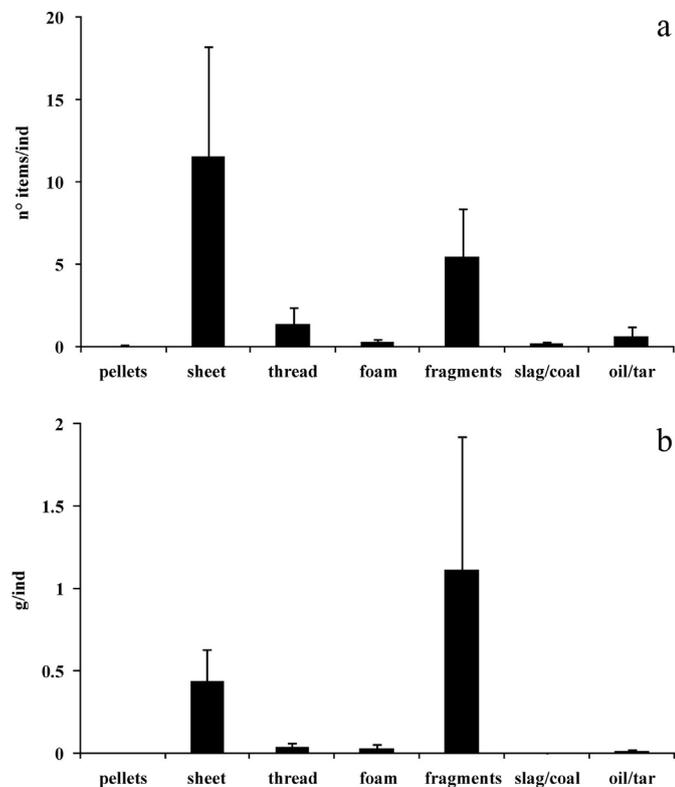


Fig. 2. Abundance and weight of marine litter ingested by turtles. On the figure “a” (abundance), it is reported the mean number of items per individual; in figure “b” (weight), it is reported the mean weight of items per individual.

**Table 5**

Frequency of occurrence of marine litter ingestion on sea turtles in the different areas. N: total sample; CCL: curved carapace length; occurrence (%): number of turtles affected by marine litter on the total sample; HL: hatchlings.

| Study area                                      | N   | CCL range (cm) | Occurrence (%) | References              |
|---|-----|----------------|----------------|-------------------------|
| <i>Mediterranean Sea</i>                        |     |                |                |                         |
| Central-western Mediterranean (Sardinia, Italy) | 121 | 21.0–73.0      | 14.0           | <i>Present study</i>    |
| Tyrrhenian sea (Tuscany coast)                  | 31  | 29.0–73.0      | 71.0           | Campani et al. (2013)   |
| Adriatic Sea (Croatia, Slovenia)                | 54  | 25.0–79.2      | 35.2           | Lazar and Gračan (2011) |
| Central Mediterranean (Italy)                   | 79  | 25.0–80.3      | 48.1           | Casale et al. (2008)    |
| Western Mediterranean (Spain)                   | 54  | 34.0–69.0      | 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  | 63.0–97.0      | 10             | Bugoni et al. (2001)    |
| North-western Atlantic (Florida, USA)           | 50  | 4.03–5.63      | 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  | HL–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)    |

campaigns are required to better understand the nature of this occurrence in Mediterranean.

The MSFD monitoring methodology for plastic presence in the marine environment will permit us to compare environmental and biological data, and to evaluate their relationship, by improving the employment of *C. caretta* as “plastic sampler” of litter in the marine environment at regional scale.

## 5. Conclusions

Anthropogenic refuses constitute a serious damage for marine ecosystems, above all for marine endangered species such as

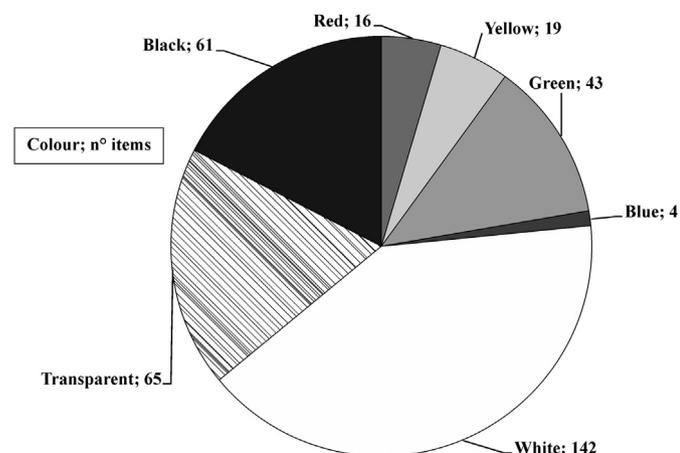


Fig. 3. Pie chart of the litter colours distribution. Litter items ingested ( $n = 333$  items) are subdivided in different colour categories (number of items).

marine turtles. Interaction of marine litter with biota needs a systematic monitoring at National and Mediterranean level by Institutions, Rescue Centres and Research Institutes. For this reason the Sardinian Network of coastal observatories for the steady monitoring of environmental conditions requires to establish a strong link among different regions. The loggerhead turtle could be considered as a sentinel of the marine environmental status, and an efficient bio-indicator of various anthropogenic pressures on the coastal environment. The providing of data on marine litter ingestion by sea turtles from all the Mediterranean countries is an ambitious project. The dialogue with RAC/SPA would allow to carry on the MSFD monitoring objectives in the entire Mediterranean basin, even in the non-European countries. The Mediterranean Network of Rescue Centres has been planned to standardize methods of protection and conservation of sea turtles, to make data comparable, and to apply common mitigation measures in order to achieve a common purpose. Data reveal that Sardinia is an important study area for its strategic location in the middle of Western Mediterranean basin and the retrieving of *C. caretta* individuals contributes to add information on the ecology and behaviour of this species. We encourage the use of *C. caretta* to monitor litter presence in the Mediterranean, as it has been for Fulmar in the North Sea (Matiddi et al., 2011; van Franeker et al., 2011). The abundance of plastics in stomachs of northern fulmars from the North Sea is indeed used in the OSPAR Ecological Quality Objective (EcoQO) for marine litter (OSPAR, 2010) and proposed for MSFD (Galgani et al., 2013; van Franeker et al., 2011). The EcoQO should be evaluated also with sea turtles when more data from all the Mediterranean area (sub-regions) will be available.

## Acknowledgements

This study has been realized within the Regional Network for Marine Turtles Conservation project and Sinis Rescue Center activity. We are grateful to “Assessorato della difesa dell’ambiente – Servizio tutela della natura” of the Sardinian Regional Body. We would like to thank especially “Capo Carbonara”, “Tavolara-Punta Coda Cavallo” Marine Protected Areas, “La Maddalena” National Park, and Vigilance Bodies. We are grateful to fishermen, tourists and citizens that collaborate with us for the retrieving of sea turtles. We are grateful to all the staff of “Istituto Zooprofilattico di Oristano” for the collaboration and the basic support during necropsies. We would like to thank the two anonymous reviewers for their useful advices and Dr. Alessandra Marra for the English revision.

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