Environmental Pollution 230 (2017) 199–209

Contents lists available at ScienceDirect

Environmental Pollution

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

Loggerhead sea turtles (*Caretta caretta*): A target species for monitoring litter ingested by marine organisms in the Mediterranean Sea☆

Marco Matiddi [a,*], Sandra Hochsheid [b], Andrea Camedda [c], Matteo Baini [d], Cristiano Cocumelli [e], Fabrizio Serena [f], Paolo Tomassetti [a], Andrea Travaglini [b], Stefano Marra [c], Tommaso Campani [d], Francesco Scholl [e], Cecilia Mancusi [f], Ezio Amato [a], Paolo Briguglio [g], Fulvio Maffucci [b], Maria Cristina Fossi [d], Flegra Bentivegna [c], Giuseppe Andrea de Lucia [c]

[a] *Italian National Institute for Environmental Protection and Research (ISPRA), Via Vitaliano Brancati 48, 00144 Roma, Italy*
[b] *Stazione Zoologica Anton Dohrn, Villa Comunale 1, 80121 Naples, Italy*
[c] *Institute for Coastal Marine Environment-National Research Council (IAMC-CNR), loc. Sa Mardini, 09170 Oristano, Italy*
[d] *University of Siena, Department of Life Sciences, San Miniato Via Aldo Moro 4, 53100 Siena, Italy*
[e] *Istituto Zooprofilattico Sperimentale del Lazio e Toscana (IZSLT), Via Appia Nuova 1411, 00178 Roma, Italy*
[f] *Tuscany Regional Agency for the Environmental Protection (ARPAT), Via Giovanni Marradi, 114, 57125 Livorno, Italy*
[g] *Clinica Veterinaria Duemari, Via Cagliari 313, 09170 Oristano, Italy*

ARTICLE INFO

Article history:
Received 14 September 2016
Received in revised form
16 June 2017
Accepted 17 June 2017

Keywords:
Marine litter
Sea turtles
Good Environmental Status
Plastic ingestion
Mediterranean Sea

ABSTRACT

Marine litter is any persistent, manufactured or processed solid material discarded, disposed of or abandoned in the marine and coastal environment. Ingestion of marine litter can have lethal and sub-lethal effects on wildlife that accidentally ingests it, and sea turtles are particularly susceptible to this threat. The European Commission drafted the 2008/56/EC Marine Strategy Framework Directive with the aim to achieve a Good Environmental Status (GES), and the loggerhead sea turtle (*Caretta caretta*, Linnaeus 1758) was selected for monitoring the amount and composition of litter ingested by marine animals. An analogous decision has been made under the UNEP/MAP Barcelona Convention for the protection of the Mediterranean Sea, following the Ecosystem Approach. This work provides for the first time, two possible scenarios for the Marine Strategy Framework Directive GES, both related to "*Trends in the amount and composition of litter ingested by marine animals*" in the Mediterranean Sea. The study validates the use of the loggerhead turtle as target indicator for monitoring the impact of litter on marine biota and calls for immediate use of this protocol throughout the Mediterranean basin and European Region. Both GES scenarios are relevant worldwide, where sea turtles and marine litter are present, for measuring the impact of ingested plastics and developing policy strategies to reduce it. In the period between 2011 and 2014, 150 loggerhead sea turtles, found dead, were collected from the Italian Coast, West Mediterranean Sea Sub-Region. The presence of marine litter was investigated using a standardized protocol for necropsies and lab analysis. The collected items were subdivided into 4 main categories, namely, IND-Industrial plastic, USE-User plastic, RUB-Non plastic rubbish, POL-Pollutants and 14 sub-categories, to detect local diversity. Eighty-five percent of the individuals considered ($n = 120$) were found to have ingested an average of 1.3 ± 0.2 g of litter (dry mass) or 16 ± 3 items.

1. Introduction

1.1. *Marine litter in the environment*

Marine litter includes all items that have been made or used by

☆ This paper has been recommended for acceptance by Eddy Y. Zeng.

* Corresponding author.
E-mail address: marco.matiddi@isprambiente.it (M. Matiddi).

http://dx.doi.org/10.1016/j.envpol.2017.06.054
0269-7491/

people and are directly or indirectly discarded in the sea. It includes those items transported from inland areas to the sea via rivers, drainage or through sewage systems, and those transported by the wind. These are materials of various origins, usages and composition, where plastics usually represent the main component (Galgani et al., 2013; MSFD TS-ML, 2011).

Waste pollution is a hazard for the entire marine ecosystem. Marine litter may harm marine animals in different ways: many species are entangled by macro litter, while other species can ingest smaller items accidentally (Laist, 1987; Schuyler et al., 2014a,b). In certain cases, ingestion occurs because marine species are unable to discriminate marine litter, while in other cases they confuse items with prey (Barnes et al., 2009; Derraik, 2002; Mrosovsky, 1981; Schuyler et al., 2014b). According to the recent works of Kühn et al. (2015, 2016), more than 500 animals are threatened by litter, including planktonic organisms (de Lucia et al., 2014; Fossi et al., 2012), sea bird species (Spear et al., 1995; van Franeker et al., 2011), fishes (Boerger et al., 2010; Lusher et al., 2013), marine mammals (de Stephanis et al., 2013; Baulch and Perry, 2014) and all species of sea turtles listed as globally vulnerable or endangered (IUCN, 2013; Schuyler et al., 2014a). Generally, the effects of ingested plastics range from direct mortality to gastrointestinal blockage, lacerations, reduced feeding and absorption of toxic compounds (Bjorndal et al., 1994; Bjorndal, 1997; Bugoni et al., 2001; Kühn et al., 2015).

Sea turtles have been used for many years as pollution bioindicators (Foti et al., 2009; Keller et al., 2006). Generally considered as a flagship species, sea turtles are able to attract the attention of different social groups and increase awareness of bad habits that affect marine environments (Frazier, 2005).

According to the opinions of 35 specialists from 13 nations involved in sea turtle biology and conservation, sea turtles have been studied more than most marine fauna. Nevertheless, management actions and their evaluation are often hindered by the lack of data on turtle biology, human interactions, population status and threats (Hamann et al., 2010). In particular, their recommendations highlight the need to evaluate the impact of plastics and other marine litter. Marine litter is a global threat for biodiversity and internationally standardized methods are essential for regular monitoring of litter.

It has been estimated that more than 62 million waste items are floating in the Mediterranean (Suaria and Aliani, 2014; Suaria et al., 2016). The majority of this litter originates from land sources but it can also arrive in the Mediterranean Sea from the Atlantic Ocean, floating via the Strait of Gibraltar (Galgani et al., 2013; MSFD TS-ML, 2011). The problem of marine litter in the Mediterranean has been considered by different studies on beach litter (Gabrielides et al., 1991; Golik and Gertner, 1992), accumulation on the sea floor (Galgani et al., 1995, 2000), and floating litter (Aliani et al., 2003). In a recent work aimed to identify the accumulation zones of floating litter in the world's oceans, using numerical models (Lebreton et al., 2012), the Mediterranean Sea was found to have one of the highest concentrations of marine litter in the world.

1.2. Policy strategy

In 2008, the European Commission adopted the Marine Strategy Framework Directive (European Commission, 2008/56/EC) whose objective is to achieve Good Environmental Status (GES) by 2020, considering 11 qualitative Descriptors. Marine litter is Descriptor 10 of the Directive and GES is reached when the "*properties and quantities of marine litter do not cause harm to the coastal and marine environment*" (European Commission, 2008/56/EC; Galgani et al., 2010). One of the "Indicators" to be monitored is known as: "*Trends in the amount and composition of litter ingested by marine animals*". In 2010, following the Commission Decision on criteria and methodological standards on GES of marine waters (European Commission, 2010/477/EU), the Directorate-General for the Environment (DG ENV) established a Technical Subgroup on Marine Litter (TSG-ML) to address gaps and further develop Descriptor 10. For many years, the Northern Fulmar (*Fulmarus glacialis*, Linnaeus, 1761), has been used as a target indicator for litter ingestion by biota in the Northern part of the European Sea. The OSPAR system of Ecological Quality Objectives (EcoQO) notes that: "There should be less than 10% of Northern Fulmars having 0.1 g or more plastic in the stomach in samples of 50–100 beached fulmars from each of 5 different regions of the North Sea over a period of at least 5 years" (van Franeker, 2004; van Franeker et al., 2011). In 2011, DG ENV asked for further development of the indicator and the methods implemented in the North Sea, and adaptation to other regions. This involved the identification of additional marine species to be used as indicators in Mediterranean EU countries.

Expert researchers of the TSG-ML elaborated a number of basic requirements for biota, which can be considered for the purposes of monitoring and selecting target species (MSFD-TS, 2013), such as:

- "***Sample availability***: *Samples of a monitoring species should be available with adequate numbers of individuals over a wider span of time and space.*"

The loggerhead sea turtle (*Caretta caretta*, Linnaeus, 1758) is adopted worldwide as a bio-indicator of environmental conditions such as pollution (Foti et al., 2009; Keller et al., 2006). The loggerhead is the most abundant chelonian in the Mediterranean (Casale and Margaritoulis, 2010; Margaritoulis et al., 2003). Many injured or dead specimens are available because of the network of sea turtle Rescue Centres established in the Mediterranean (Ullmann and Stachowitsch, 2015; RAC-SPA, 2004), and the large number of accidental captures in fishing gears along the Italian coasts (Cambiè, 2011; Casale et al., 2013; de Lucia et al., 2011).

- "***Regular plastic consumption***: *Frequency of occurrence and amounts of plastic found in stomachs should be high enough to allow detection of trends over time and geographical patterns.*"

Loggerheads may ingest plastic bags mistaken for jellyfishes (Fukuoka et al., 2016; Mrosovsky, 1981; Mrosovsky et al., 2009; Plotkin et al., 1993) when they feed in neritic and pelagic habitats. The regular occurrence of solid waste in the stomach contents is documented worldwide (Bjorndal et al., 1994; Hamann et al., 2010. Hoarau et al., 2014; Lazar and Gracan, 2011; Santos et al., 2016) and along the Mediterranean coast (Bentivegna et al., 2013; Camedda et al., 2013, 2014; 2015; Campani et al., 2013; Casale et al., 2008, 2016; de Lucia et al., 2012; Matiddi et al., 2015; Travaglini et al., 2013; Tomás et al., 2002).

- "***Marine feeding habits***: *stomach contents should only reflect the marine environment. For example, many gulls ingest litter, but partially feed on land including rubbish dumps.*"

Loggerhead sea turtles feed exclusively at sea although they frequent different and disparate areas during their life, inhabiting both oceanic and neritic zones. In general, adults/sub-adults use the sea bottom and the water column as feeding compartment, while early juveniles prefer the sea surface; for these reasons, they are likely to ingest waste in different habitats during their life (Bjorndal, 1997; Casale et al., 2008; Lazar et al., 2010).

Each of the above requirements supports the use of the loggerhead sea turtle as an assessment and monitoring tool for litter ingested by marine organisms. After some considerations and a

pilot study carried out by Italian researchers, the experts of the TSG-ML nominated by the Member States, have chosen the loggerhead sea turtle, as target species for monitoring litter ingested by marine organisms in the Mediterranean Sea, like the Fulmar in the OSPAR countries (Matiddi et al., 2011; MSFD-TS, 2013).

Similar considerations have been taken into account within the framework of the Barcelona Convention LBS protocol, where a Policy Document and the associated Strategic Framework for Marine Litter management were adopted in 2012 (UNEP(DEPI)/MED, 2012). One of its main objectives is to follow monitoring programs for marine litter in the Mediterranean Sea, based on the Ecosystem Approach. In this case also, the loggerhead sea turtle has been recommended by the expert group (CORMONs pollution and litter) as the main target species regarding "Common Indicator 18E: *Trends in the amount of litter ingested by or entangling marine organisms*" (UNEP (DEPI)/MED, 2014) where entanglement has been proposed as an alternative to litter ingestion. Despite that, only few data on the distribution and density of marine litter ingested by sea turtles in the Mediterranean Sea are currently available and the protocols of analysis are not harmonized or comparable. Furthermore, the characteristics and the quantity of plastic items ingested at regional level are still unknown.

1.3. The study

This study aims to confirm the loggerhead sea turtle as the main target species in the Mediterranean Sea for monitoring litter ingested by marine organisms, similarly to the use of the Fulmar in the North Sea (Matiddi et al., 2011; van Franeker et al., 2011). The different types of items observed in the gastrointestinal tract of stranded sea turtles, were analyzed according to the "Litter in Biota" protocol included in the "Monitoring Guidance for Marine Litter in European Seas" report (MSFD-TS, 2013). Following the EcoQO for Fulmar, amounts of litter *vs* plastic ingested by sea turtles were compared and two different scenarios for GES relating to "*Trends in the amount and composition of litter ingested by marine animals*" in the Mediterranean Sea were established. The first scenario verifies the applicability of the Fulmar protocol to the loggerhead case, in order to allow harmonization of the procedure throughout European sea waters. The second scenario is a novelty since it also takes animal health into consideration; this approach should be considered when dealing with sea turtles worldwide.

This paper presents the result of a pilot project conducted by the Italian task group led by ISPRA (IAMC-CNR, SZN "Anton Dohrn", University of Siena, ARPAT) that used the loggerhead sea turtle as a target species for the evaluation of ingested marine litter, according to an experimental protocol developed specifically for the Mediterranean Sea. The results established a baseline of methodologies and considerations, which are relevant worldwide in areas where both sea turtles and marine litter are present.

2. Material and methods

2.1. Sampling activity and necropsy

From 2011 to 2014, 150 stranded loggerhead sea turtles found dead along the Italian coasts, Western Mediterranean Sea, were collected; 20 from Sardinia, 53 from Tuscany, 29 from Lazio and 48 from Campania (Fig. 1). According to Italian legislation and following agreement between the local Coast Guard and Rescue Centres of the various regions, marine wildlife, such as marine mammals and sea turtles found along the coast, are transported to the authorized centres when found dead or alive in bad conditions (ISPRA, 2013).

According to the regional classification adopted by the Marine Strategy Framework Directive, all the samples came from the same Italian sub-Region. At the Rescue Centre, each individual was measured using a flexible meter to the nearest 0.5 cm (standard curved carapace length, CCL). Visual inspection was performed in order to verify any possible causes of death or disease, including entanglement in derelict nets, longlines or plastic bags. Gastrointestinal tracts (GI) were isolated by cutting the main plastron ligament and removing the pectoral and pelvic musculature. Briefly, plastic clamps were fixed on the oesophagus, stomach and intestine before removing the GI from the carcass in order to prevent spillage or mixing of the ingested materials present in the different portions. Each portion of the GI was analyzed separately. The contents were emptied onto a 1 mm mesh sieve, visually inspected for the presence of tar, oil or any particularly fragile materials and then washed with fresh water. Items retained by the sieve were collected and preserved in 70% ethanol solution until analysis. All the materials were dried for 24 h before being sorted and analyzed under a stereo-microscope. Specimens with completely empty GIs were not included in the analysis, thus avoiding consideration of individuals that had been sick for a long time before stranding.

2.2. Ingested marine litter

Anthropogenic litter was separated from other ingested residue and categorized according to the "Litter in Biota" protocol included in the "Monitoring Guidance for Marine Litter in European Seas" report (Matiddi et al., 2011; MSFD-TS, 2013). Following the protocol, the items were subdivided into 4 main categories (IND-Industrial plastic, USE-User plastic, RUB-Non plastic rubbish, POL-Pollutants), including 14 different subcategories, plus food remains (*Foo*) and natural non food remain (*Nfo*) (Table 1). Even if some sea turtles had fishing hooks in their GI, data were not included in the analysis because, according to the protocol, fishing hooks on which longline victims are actively caught are not considered as "marine litter". Items were counted and weighed, and the dry mass of each category was obtained to the nearest 0.001 g. Marine litter items collected in the GI were also classified into 8 categories, based on their colour.

Foo, remains of the natural diet of loggerheads, and *Nfo*, natural items that cannot be considered as normal nutrition (Table 1), were only weighed.

2.3. Data analysis

Frequency of occurrence (FO), the percentage of dead animals with litter in the GI, was calculated globally and for all sampling areas. As regards the impacted turtles, the difference of FO inside the oesophagus, stomach and intestine was analyzed.

Univariate analyses of variance were performed using PRIMER 6 software (Plymouth Marine Laboratory, UK) with the PERMANOVA + package (Anderson et al., 2008), based on Euclidian distances, in order to evaluate the differences in the amount and weight of marine litter in samples from different regions. The number of litter items and their dry mass were Log (x+1) transformed. Sub-categories with contributions below the 1.5% of total dry mass threshold were not included in the analysis. Moreover, 9999 random permutations and an additional Monte Carlo test were performed to obtain a robust analysis even if the number of unique permutations was low (Anderson et al., 2008).

Univariate Anova tests were also performed, using the GMav 5 statistical software package (University of Sidney, Australia), on Log (x+1) transformed data to detect differences in the amount and weight of litter sub-categories (both on the total dataset and 'single region' data) and among different litter colours (only on the total dataset). As the number of degrees of freedom was high (up to 40

Fig. 1. Study area.

Table 1
Marine litter ingested by sea turtles per category and sub-category according to *Litter in Biota*: *Guidance on Monitoring of Marine Litter in European Seas* (MSFD-TS, 2013).

Typology	Category	Sub-category	Description
	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.
PLASTIC	User plastics (USE)	*Sheet (she)*	Remains of sheet, e.g. from bags, 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 such as 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 little bit flexible, but not like sheet-like materials.
		Other (Poth)	Any other, including elastics, dense rubber, cigarette filters, balloon pieces, soft airgun bullets.
NON PLASTIC RUBBISH	Non plastic rubbish (RUB)	*Paper (pap)*	Newspaper, packaging, cardboard, including multilayered material (e.g. Tetrapack pieces) and aluminium foil.
		Kitchen food (kit)	Human food remains (galley wastes) such as onion, beans, chicken bones, bacon, tomato seeds, grapes, peppers, melon, etc.
		Other user (rva)	Other consumer waste, such as processed wood, pieces of metal, metal air-gun bullets; lead shot, paint chips.
		Fishhook (hoo)	Fishing hook remains (not for hooks on which longline victims were caught).
POLLUTANTS	Pollutants (POL)	*Slag/coal (sla)*	Industrial oven slag (looks like non-natural pumice) or coal remains.
		Oil/tar (tar)	Lumps of oil or tar
		Paraf/chem (che)	Lumps or mush of unclear paraffin, was like substances (not stomach oil!) if needed subsample and estimate mass.
FOOD	Natural Food	*Foo*	Remains of the natural diet of the animal such as otoliths, fish bones and spines, parts of crustaceans, molluscs, jellyfishes, etc.
	Natural Non Food	*Nfo*	Anything natural, but which cannot be considered as normal nutrition for the individual.

df), it was not necessary to test for homogeneity of variance (Benedetti-Cecchi, 2003; Underwood, 1997). In case of significant differences, post-hoc Student-Newman-Keuls (SNK) Tests were ran. A regression analysis using Spearman's rank correlation was performed to determine the relationship between sea turtle length (CCL) and marine litter dry mass or number of litter items.

Amounts of litter *vs* plastic ingested by sea turtles were compared and 2 different scenarios for GES in the Mediterranean Sea were established:

- First scenario: *"There should be less than X% of* loggerheads *having Y g or more plastic in the stomach in samples of 50-100 stranded loggerheads from each sub-region"*, where Y is the average value of plastic ingested and X% the percentage of sea turtles with more grams of plastic than Y.
- Second scenario: *"There should be less than X% of* loggerheads *having more plastic grams than food remains (Foo) in the stomach in samples of 50-100 stranded loggerheads from each sub-region"*, where there is no fixed value. Plastic grams are compared with food remain for each loggerhead.

According to a number of authors, juvenile loggerheads feed mainly on planktonic organisms (e.g. cnidaria or ctenophora) and begin to feed on benthic prey (e.g. crustaceans, sponges, molluscs) during growth (Bjorndal, 1997; Frick et al., 2009; Lazar et al., 2010). For this reason, early juvenile turtles (CCL $\leq$40 cm) were distinguished from sub-adult and adult ones, in the GES analysis.

3. Results

Turtle CCL ranged from 21.0 to 82.7 cm, with an average of 60.6 cm ($n = 150$), and there were 16 early juveniles (CCL $\leq$40 cm). Out of the 48 sea turtles without ingested marine litter, 30 specimens with a completely empty GI (0 g of litter, 0 g of natural food

remain, 0 g of natural non food remain) were excluded from the analysis (Table 2).

We did not find any sea turtles that were seriously damaged due to blockage of the digestive tract, while only two cases of entanglement were recorded.

Frequency of occurrence was calculated for the entire sample (n = 120; FO = 85%) and for the different sampling areas (Lazio n = 26 FO = 100%; Tuscany n = 39 FO = 97%; Sardinia n = 9 FO = 89%; Campania n = 46 FO = 65%). In total, 157.243 g (dry mass) and 1962 items (abundance) of marine litter were collected with an average value of 1.3 ± 0.2 g and 16 ± 3 items.

Marine litter was detected mainly in the intestine (FO = 75%; total mass 127.889 g) followed by the stomach (FO = 32%; total mass 27.845 g), while the oesophagus was the least affected part of the GI (FO = 5%; total mass 1.508 g). No specimens with marine litter in the oesophagus only were found and there was only one case with marine litter in the oesophagus and absence in the intestine.

Among the impacted turtles (n = 102), parts of plastic bags and other sheet fragments (USE *she*) were the main categories in terms of abundance (12±2items; $p < 0.01$), followed by rigid plastic fragments (USE *fra* 3.0 ± 0.5 items) and threads (USE thr 2±1items) (Fig. 2A). USE *she* was also the main category for mass together with USE *fra* (USE she 0.5 ± 0.1 g; USE *fra* 0.6 ± 0.1 g; $p < 0.01$) (Fig. 2B). Raw plastic pellets or other kinds of industrial plastic were not found in our samples, while each "Non-plastic" sub-category did not reach the limit of 1.5% of total items, in terms of mass or abundance.

At local level, USE *she* remained the main category in each region, in terms of abundance (Lazio n = 26, 14 ± 6 items; Tuscany n = 38, 13 ± 4 items; Campania n = 29, 9±3items; $p < 0.01$), but in Sardinia the difference between USE *she* and USE *fra* was not significant (n = 9, USE *she* 5 ±2items; USE *fra* 2±1items) (Fig. 3A). For dry mass, USE *she* was the main category in each Region except Tuscany, whereas USE *fra* had a slightly higher mean value of dry mass (USE *she* 0.7 ± 0.3 g; USE *fra* 1.1 ± 0.4 g). In Lazio, USE *she* did not differ significantly from USE *fra* and USE *thr* (USE she 0.6 ± 0.2 g, USE *fra* 0.3 ± 0.1 g, USE *thr* 0.4 ± 0.3 g), while in Campania and Sardinia it did not differ from USE *fra* (USE *she* 0.4 ± 0.1 g, USE *fra* 0.4 ± 0.2 g Campania; USE *she* 0.2 ± 0.1 g, Use *fra* 0.2 ± 0.1 g Sardinia) (Fig. 3B).

The factor 'Region' (area) was significant for samples from Tuscany and Lazio, being the most polluted zones, as regards abundance and dry mass, while Sardinia showed the lowest value. Tuscany and Lazio showed similar values (Tuscany 20±3items; Lazio 20 ± 4 items), both significantly higher than the values detected in Campania (13 ± 2 items) ($p < 0.01$). Samples from Sardinia (9 ± 3 items) did not differ from those of the other regions probably due to high variability of the sample. Even when dry mass was considered (Tuscany 2. 1 ± 0.6 g; Campania 0.8 ± 0.2 g; Lazio 1.4 ± 0.5 g; Sardinia 0.4 ± 0.2 g), the factor region was significant ($p < 0.05$). In particular, a significant difference was detected between the values of Tuscany and Campania. The maximum value for grams (17.360 g) and abundance (200 items) was found in two specimens, both collected in Campania.

The mean abundance of ingested anthropogenic materials differed significantly between colour categories. Transparent objects (8±2items; $p < 0.01$) were the main item ingested by sea turtles in our samples, followed by white (4 ± 1 items) and black (3 ± 1 items) (Fig. 4).

Ingestion of marine litter was observed for both growth stages, early juveniles (FO 100%, n = 10) and adults/sub-adults (FO 83% n = 110), but regression analysis showed that there was no correlation between CCL *vs* grams (Spearman correlation = 0.08) or CCL *vs* number of items (Spearman correlation = 0.04).

Due to the scarce number of early juveniles CCL≤40 cm (n = 10), it was decided to elaborate the GES scenarios for sea turtles with CCL > 40 cm (n = 110) only:

Scenario 1 *"There should be less than 27% of loggerheads having 1.3 g or more plastic in the stomach in samples of 110 stranded loggerheads from the Western Mediterranean sub-Region."*

Scenario 2 *"There should be less than 64% of loggerheads having more plastic grams than food remains (Foo) in the stomach in samples of 110 stranded loggerheads from the Western Mediterranean sub-Region."*

4. Discussion

4.1. Good Environmental Status

The outcome of the analysis of the first scenario, based on 110 adult/sub-adult loggerheads (CCL>40 cm) with a mean value of 1.3 ± 0.2 g, is in line with the UNEP/MAP proposed baseline (1–3 g) (UNEP (DEPI)/MED 2015; 2016). Moreover, in this scenario 27% of the sampled loggerheads presented values of litter than were over the mean value found for the entire sample set; this is a high value even in the context of a highly polluted area such as the one under study. Therefore, to overcome this and to reach GES by 2020, it would be necessary to reduce the amount of litter in the environment or improve mitigation actions in the area. This result confirms that the loggerhead is a good indicator of the "*Trends in the amount and composition of litter ingested by marine animals*" (European Commission, 2010/477/EU) for the Mediterranean area, as is the fulmar for the North Sea. However, it is important to bear in mind that the Mediterranean Sea is a highly polluted area (Suaria and Aliani, 2014; Lebreton et al., 2012) when comparing the results with those for the fulmar, where less than 10% of the individuals could exceed the fixed threshold (0.1 g; van Franeker, 2004).

In the second scenario, "harm" (Werner et al., 2016) was also detected as a possible impact, since the weight (grams) of food remains (*Foo*) and plastic were compared. This should be done

Table 2
Sample size used in the different analyses. Total: All the collected stranded turtles. These data were not considered in the analysis, including 30 specimens with a completely empty GI; Impacted: turtles with ingested marine litter in the GI; Not impacted: turtles with ingested food remains (Foo) or natural non food remains (Nfo) but without litter in the GI; GES: turtles with CCL longer than 40 cm, used in both GES scenarios. *110 adults and 10 early juveniles. **92 adults and 10 early juveniles.

	Sample size	Incidence (FO%)	Total number of items average ± s.e.	Total mass average ± s.e. (g)	Plastic number of items average ± s.e.	Plastic mass average ± s.e. (g)
Total	150	68%	13 ± 2	1.0 ± 0.2	12 ± 2	1.0 ± 0.2
impacted + not impacted	120*	85%	16 ± 3	1.3 ± 0.2	15 ± 3	1.2 ± 0.2
Impacted	102**	100%	19 ± 3	1.5 ± 0.3	18 ± 3	1.4 ± 0.3
GES	110	83%	17 ± 3	1.4 ± 0.2	16 ± 3	1.3 ± 0.2

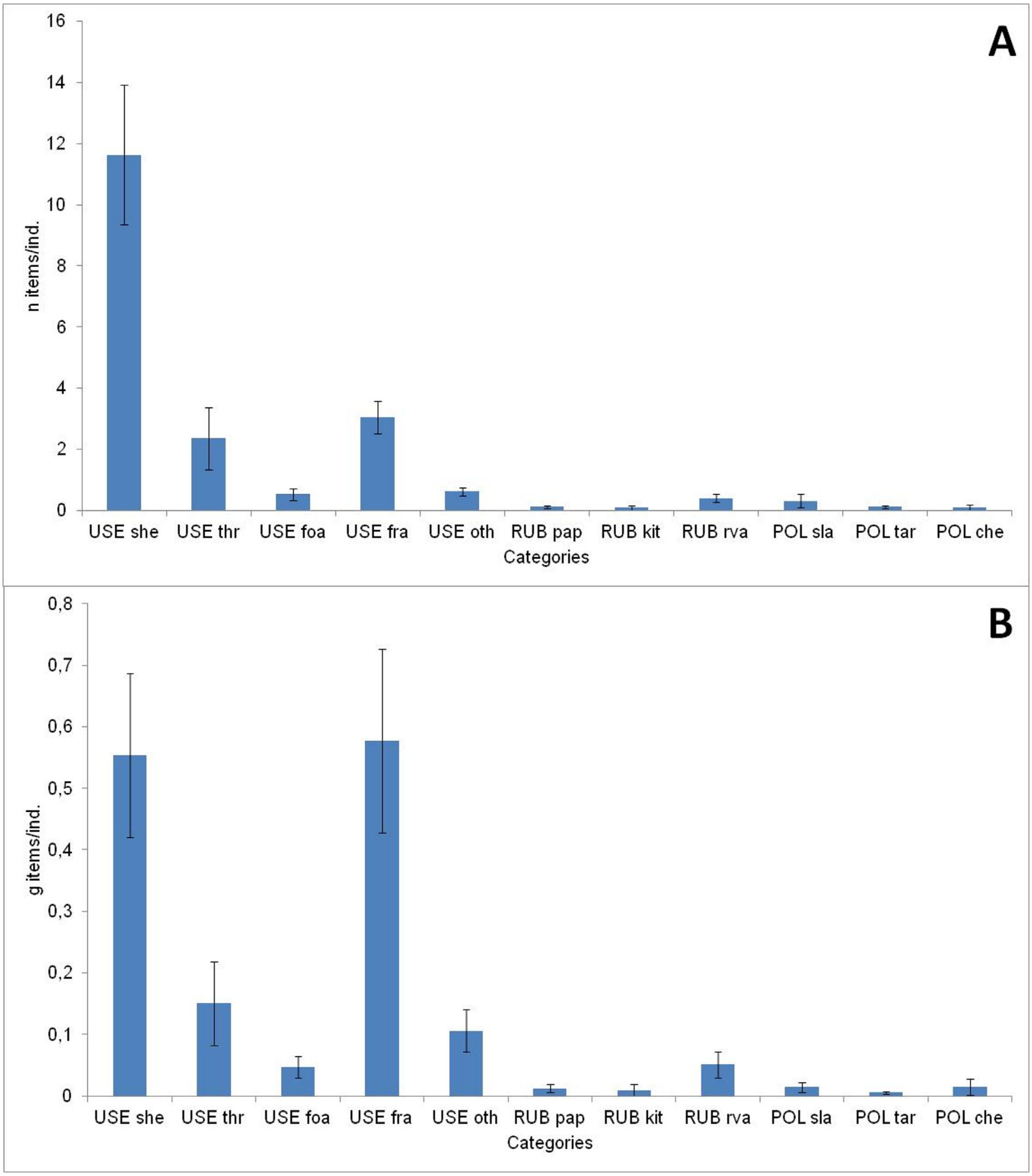

Fig. 2. Abundance and weight of marine litter categories ingested by turtles. Figure "A" shows the mean number of items per individual; figure "B" presents the mean weight, in grams, of items per individual.

individually for each turtle analysed, since the result is strongly related to the presence of food ingested, thus indicating that the individual is in good or bad health condition. According to our results, 64% of the individuals exceeded the threshold, having more grams of plastic than food remains in their GIs. This must be considered as serious harm to the animal caused by marine litter. The methodology used in the second scenario should be taken into consideration when analysing the impact of litter on biota worldwide. To reduce this negative result it is urgent to implement effective mitigation policies that aim to reduce the amount of litter in the environment. The Marine Strategy Framework Directive aims to achieve Good Environmental Status (GES) in the EU's marine waters by 2020 and thus protect the resources on which economic activities are based and the welfare of society. If we follow the EcoQO (OSPAR), the GES value should also be based on data obtained from the least polluted areas in the Mediterranean. These areas have not yet been defined and it is therefore difficult to establish GES for the entire Mediterranean area. Here we present a baseline for defining GES for the Western Mediterranean sector, a highly polluted area, based on the interaction between loggerheads and litter.

The MSFD directive and the EcAp framework have already

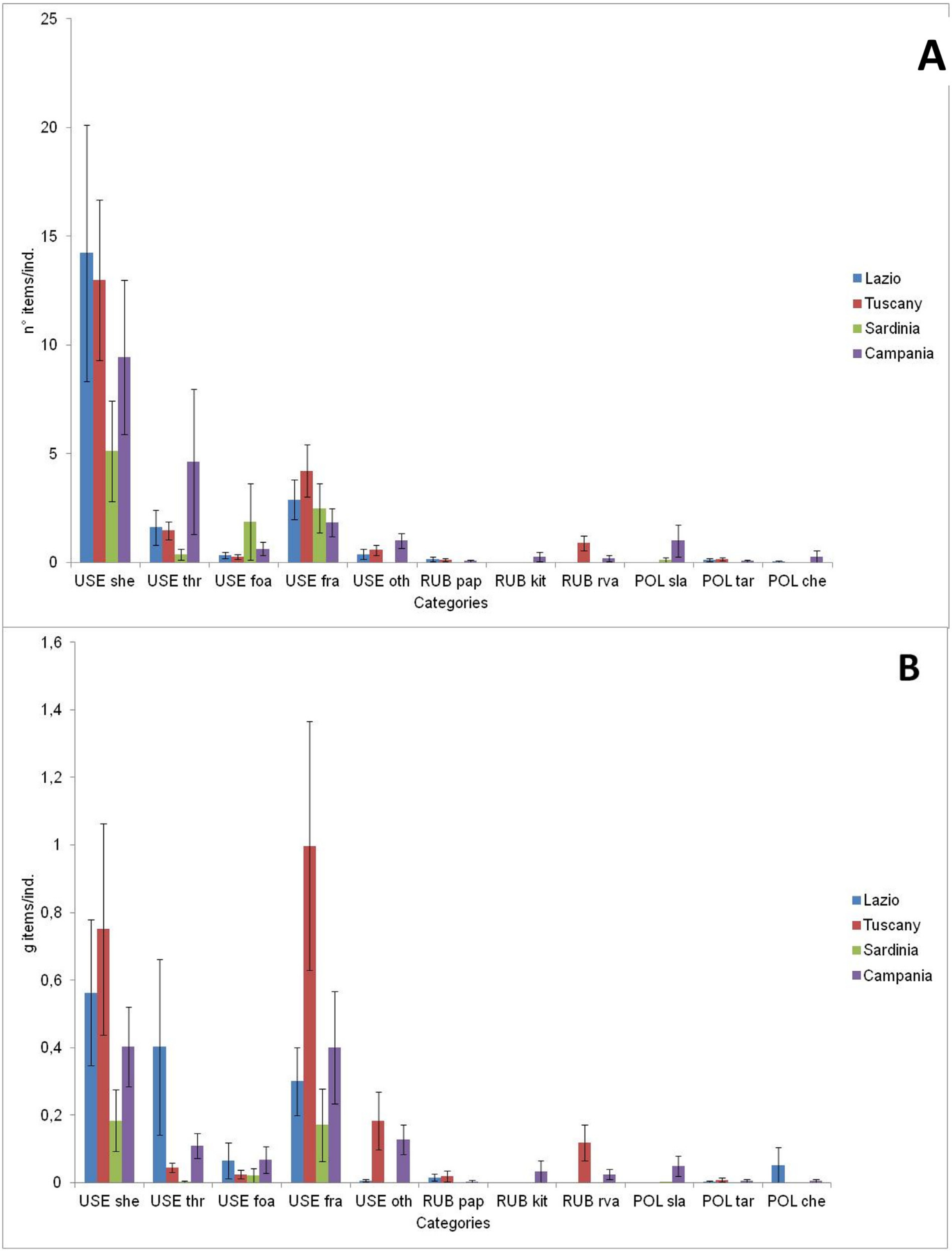

Fig. 3. Abundance and weight of marine litter categories ingested by turtles in the different Italian Regions (Lazio, Tuscany, Sardinia, Campania). Figure "A" shows the mean number of items per individual; figure "B" presents the mean weight of items per individual.

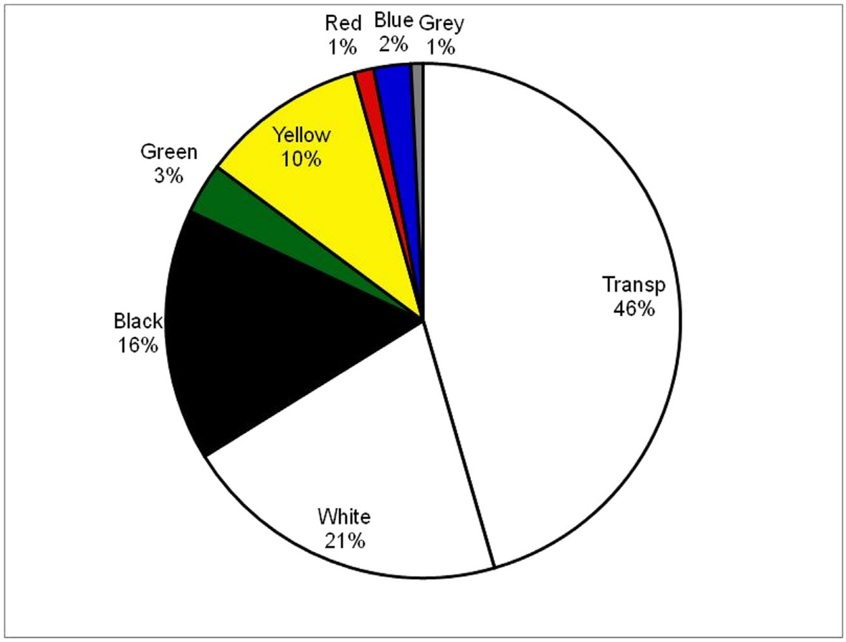

Fig. 4. Abundance of litter items ingested, subdivided into colour categories (number of items); the percentage of each category with respect to the total amount are shown in the figure. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

created work groups that are establishing a baseline on which future decision processes will be proposed by researchers and stakeholders and, therefore, improving efficiency at national and international level. This approach should be inspired by other types of cooperation that are taking place at European level. For example, the European Environmental Agency, within the driver-pressure-state-impact-response (DPSIR) framework, is providıng accessible information and links between policy-makers and researchers (Gabrielsen and Bosch, 2003).

As pointed out by Nicholson et al. (2009), to understand the human dimension of the threats, they should not be related to sea turtles only but also to ecosystem services and economic functions. In this context, the first and the second scenario provide a useful tool for the assessment of marine litter pressures and impacts and thus improving the guidelines for developing policies and conservation actions.

According to the MSFD, "Targets" must be adopted for the above Indicators, in order to achieve GES by 2020. The values of these targets should be the significant reduction of values obtained for the two scenarios. This would be the result of effective measures taken by Member States to reduce the amount of marine litter and achieve or maintain GES. The results of the current study are specific to the Western Mediterranean sector, although the methodology can be adopted worldwide for wider study areas as well as for different sea turtle species.

4.2. Ingestion rate

This study highlights that most of the analyzed individuals had ingested plastic particles, thus contributing to a high frequency of occurrence in the entire sample. Our data show a FO of 85%, higher than any other value previously reported in the Mediterranean Region (Gramentz, 1988; Tomás et al., 2002; Lazar and Gracan, 2011; Campani et al., 2013; Camedda et al., 2014; Casale et al., 2016) or in the Oceans (Plotkin and Amos, 1988; Bugoni et al., 2001; Frick et al., 2001, 2009; Plotkin et al., 1993; Witherington, 1994; Parker et al., 2005; Boyle and Limpus, 2008; Hoarau et al., 2014; Nicolau et al., 2016). Our value is also much higher than the marine litter baseline proposed by UNEP/MAP, i.e. 40–60% (UNEP (DEPI)/MED, 2015; 2016). Frequency of occurrence has often been considered in previous studies on sea turtle litter ingestion worldwide, and the values observed had a great range of variation, depending on different factors, such as study area, number of samples, seasonality, etc. The differences in our FO compared to others studies could be linked not only to the highly polluted area considered in our study (Còzar et al., 2015; Suaria et al., 2016) but also to the different methodologies used by others.

We only considered sea turtles that had ingested something ($n = 120$), excluding specimens with a completely empty GI ($n = 30$). Using this method, we excluded animals unable to feed, considered sick for a long time before being stranded. If we include these animals in the data, we obtain a significant reduction of FO%, from 85% to 68% (Table 2), more or less in line with other studies. In any case, Fukuoka et al. (2016) showed similar FO% in loggerhead turtles from Japanese waters (84.6%), but this value concerns a different number of specimens.

4.3. Local scale

This is the first study based on a standardized protocol that quantifies litter ingested by sea turtles in four different areas of the Western Mediterranean sub-Region.

The analyses allowed to detect different pollution levels at local scale (factor Region $p < 0.05$) and, as assumed by Casale et al. (2016), stranded turtles are more representative of coastal/neritic turtles than of pelagic ones. Nevertheless, this result should be considered with caution as sea turtles are migratory species that travel long distances, quantified in dozens of kilometres per day (Bentivegna, 2002; Bentivegna et al., 2007; Schofield et al., 2010; Tucker, 2010; Varo-Cruz et al., 2013). Studies on the transit time of substances in the gastro-intestinal tracts of loggerhead sea turtles have demonstrated that these materials are expelled in about 10 days (Camedda et al., 2014; Valente et al., 2008). According to us, more data relating to animal resident time in a specific area are

needed to locate the exact source of pollution at local scale.

No significant differences were observed in terms of composition of ingested debris among different areas. Plastic items are the most frequent litter sub-categories found in our samples (around 90% of total ingestion), in agreement with other studies (Tomás et al., 2002; Casale et al., 2008; Camedda et al., 2014). On the contrary, non-plastic litter sub-categories did not reach the limit of 1.5%. Therefore, we suggest simplification of the protocol by pooling "Non-plastic rubbish" and "Pollutants" into one typology (e.g. "Other").

4.4. Turtle ecology

Loggerheads can ingest marine litter by confusing it with typical diet items or by accidental ingestion when consuming natural prey. Floating plastic bags are reported to be mistaken for prey by sea turtles (Plotkin et al., 1993; Casale et al., 2008; Mrosovsky et al., 2009; Fukuoka et al., 2016) and jellyfishes are more likely to be found, as "*sheet*" items, in the middle of the water column (Schuyler et al., 2014a,b). The ingestion of plastic items could also be a consequence of the active predation of sea turtles on the epibiontes that colonize them, both on the bottom and in the water column. In some cases it has been shown that turtles change travelling direction and reduce swimming speed in order to bite litter but they are also able to distinguish, as described by Narazaki et al. (2013) and Fukuoka et al. (2016). Nevertheless, we do not consider our results (transparent + white sheet = 42.7%) as proof of predation by turtles on plastic, due to the lack of data on marine litter sub-category amounts and distribution in the Mediterranean Sea. The high percentage of sheet items may reflect the predominance of this sub-category as floating marine litter along the Italian coast, as suggested by Nicolau et al. (2016) for the Portuguese coast. Moreover, transparent soft plastic has been found to be the most observed item along the Japanese coast (Fukuoka et al., 2016).

According to our findings (only two cases of entanglement were recorded during this study), we consider ingestion as the main interaction between sea turtles and marine litter. Furthermore, we found most of the litter items in the last tract of the gut (intestine 81% of dry mass), close to be excreted. This confirms that the generalist feeding strategy of loggerheads seems to place them at high risk of ingesting plastic. But loggerheads are also able to eliminate plastic items through defecation, thus demonstrating a certain degree of tolerance as shown in previous studies (Tomás et al., 2002; Hoarau et al., 2014; Nelms et al., 2015; Fukuoka et al., 2016).

This finding suggests that litter ingestion produces mostly sub-lethal effects rather than causes death as suggested by Nelms et al. (2015) and Nicolau et al. (2016). In addition, this demonstrates that the intestine should be the main part of the GI to be examined during a necropsy, while an examination of the oesophagus and stomach only, would underestimate litter abundance.

Age was found to influence litter quantities in fulmar stomach contents (van Franeker and Meijboom, 2002). In sea turtles, age is strongly related with CCL (Bjorndal, 1997; Frick et al., 2009; Lazar et al., 2010) and our sample did not show any kind of relation between CCL *vs* litter ingestion, in line with previous studies (Camedda et al., 2014; Nicolau et al., 2016).

5. Conclusion

The Integrated Monitoring and Assessment Programme (IMAP) including 23 Common Indicators and 4 Candidate Indicators was adopted at the 19th Meeting of the Parties to the Barcelona Convention in February 2016 (UNEP(DEPI)/MED, 2017). In this framework, our scenarios represent a useful tool for the detection of "*Trends in the amount of litter ingested by marine animals*".

We strongly encourage the use of *C. caretta* as target species to monitor litter presence in Mediterranean countries (*sensu* MSFD and Barcelona Convention), as the fulmar has been used as indicator species in the Northern European Sea. We suggest a pilot action for the application of the "Litter in Biota" protocol (Matiddi et al., 2011; MSFD-TS, 2013) throughout the Mediterranean Basin under the coordination of UNEP/MAP and/or the Directorate General for the Environment of the European Commission. Harmonization of results will allow large-scale testing of the two scenarios elaborated in this study for GES and quantification of spatial and temporal trends. Urgent mitigation policies and a Programme of Measures are needed and should be developed at national and international level.

Acknowledgements

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors. We are grateful to the Italian Coast Guard, fishermen, tourists and citizens who collaborated with us for retrieving sea turtles.

We would like to thank Dr. Francesca Pau and Dr. Manuela D'Amen for their feedback.

References

Aliani, S., Griffa, A., Molcard, A., 2003. Floating debris in the Ligurian sea, north-western mediterranean. Mar. Pollut. Bull. 46, 1142–1149.
Anderson, M.J., Gorley, R.N., Clarke, K.R., 2008. PERMANOVA for PRIMER: Guide to Software and Statistical Methods. PRIMER-E, Plymouth, UK.
Barnes, D.K.A., Galgani, F., Thompson, R.C., Barlaz, M., 2009. Accumulation and fragmentation of plastic debris in global environments. Philos. Trans. R. Soc. B 364, 1985–1998. http://dx.doi.org/10.1098/rstb.2008.0205.
Baulch S., and Perry C., 2014. Evaluating the impacts of marine debris on cetaceans. 80 (1–2), 210–221.
Benedetti-Cecchi, L., 2003. In: Gambi, M.C., e Dappiano, M. (Eds.), Disegno sperimentale ed analisi di ipotesi in ecologia. Manuale di metodologie di campionamento e studio del benthos marino mediterraneo, vol. 13. Società italiana di Biologia marina, pp. 433–484.
Bentivegna, F., 2002. Intra-Mediterranean migrations of loggerhead sea turtles (Caretta caretta) monitored by satellite telemetry. Mar. Biol. 141, 795–800.
Bentivegna, F., Valentino, F., Falco, P., Zambianchi, E., Hochscheid, S., 2007. The relationship between loggerhead turtle (Caretta caretta) movement patterns and Mediterranean currents. Mar. Biol. 151, 1605–1614.
Bentivegna, F., Travaglini, A., Matiddi, M., Baini, M., Camedda, A., De Lucia, A., Fossi, M.C., Giannetti, M., Mancusi, C., Marchiori, E., Poppi, L., Serena, F., Alcaro, L., 2013. First data on ingestion of marine litter by loggerhead sea turtles, Caretta caretta, in Italian waters (Mediterranean sea). In: Proceedings of the Biology and Ecotoxicology of Large Marine Vertebrates and Sea Birds: Potential Sentinels of Good Environmental Status of Marine Environment, Implication on European Marine Strategy Framework Directive. 5–6 June, Siena Italy.
Bjorndal, K.A., 1997. Foraging ecology and nutrition of sea turtles. In: Lutz, P., Musick, J. (Eds.), The Biology of Sea Turtles, vol. 1. CRC Press, Boca Raton, FL, pp. 199–232.
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.
Boerger, C.M., Lattin, G.L., Moore, S.L., Moore, C.J., 2010. Plastic ingestion by planktivorous fishes in the North Pacific central gyre. Mar. Pollut. Bull. 60, 2275–2278.
Boyle, M.C., Limpus, C.J., 2008. The stomach contents of post-hatchling green and loggerhead sea turtles in the southwest Pacific: an insight into habitat association. Mar. Biol. 155, 233–241.
Bugoni, L., Krause, L., Petry, M.V., 2001. Marine debris and human impacts on sea turtles in Southern Brazil. Mar. Pollut. Bull. 44, 842–852.
Cambiè, G., 2011. Incidental capture of Caretta caretta in trammel nets off the western coast of Sardinia (Italy): statistical models of capture abundance and immediate survival. Aquat. Conserv. Mar. Freshw. Ecosyst. 21, 28–36.
Camedda, A., Matiddi, M., Massaro, G., Coppa, S., Perilli, A., Ruiu, A., Briguglio, P., de Lucia, G.A., 2013. Five years data on interaction between loggerhead sea turtles and marine litter in Sardinia. In: Proceedings of the Biology and Ecotoxicology of Large Marine Vertebrates and Sea Birds: Potential Sentinels of Good Environmental Status of Marine Environment, Implication on European Marine Strategy Framework Directive. 5–6 June, Siena.
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.

Camedda, A., Marra, S., Matiddi, M., Massaro, G., Coppa, S., de Lucia, G.A., 2015. An increasing trend on the interaction between loggerhead sea turtle (Caretta caretta) and marine litter in Sardinia (Western mediterranean sea). In: Proceedings of the 35th Annual Symposium on Sea Turtles Biology and Conservation. 19–24 April, Dalaman Turkey.
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, 1330–1334.
Casale, P., Margaritoulis, D., 2010. Sea Turtles in the Mediterranean: Distribution, Threats and Conservation Priorities. IUCN, Gland, Switzerland.
Casale, P., Abbate, G., Freggi, D., Conte, N., Oliviero, M., Argano, R., 2008. Foraging ecology of loggerhead sea turale 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., Cina', A., Rocco, M., 2013. Spatio-temporal distribution and migration of adult male loggerhead sea turtles (Caretta caretta) in the Mediterranean Sea: further evidence of the importance of neritic habitats off North Africa. Mar. Biol. 160, 703–718. http://dx.doi.org/10.1007/s00227-012-2125-0.
Casale, P., Freggi, D., Paduano, V., Oliverio, M., 2016. Biased and best approaches for assesting 527 debris ingestion in sea tirale, with a case study in the Mediterranean. Mar. Pollut. Bull. 110 (1), 238–249. http://dx.doi.org/10.1016/j.marpolbul.2016.06.057.
Còzar, A., Sanz-Martín, M., Martí, E., González-Gordillo, J.I., Ubeda, B., Gálvez, J.A., Irigoien, X., 2015. Plastic accumulation in the mediterranean sea. PLoS One 10, e0121762.
de Lucia, G.A., Massaro, G., Magnone, F., Fracassi, D., Frau, F., Gaio, A., Ollano, G., Piereddu, L., Secci, E., 2011. The marine species conservation network of sardinia (marine turtles and mammals). Biol. Mar. Mediterr. 18 (1), 154–155.
de Lucia, G.A., Matiddi, M., Travaglini, A., Camedda, A., Bentivegna, F., Alcaro, L., 2012. Marine litter ingestion in loggerhead sea turtles as indicator of floating plastic debris along Italian coasts. In: Proceedings of the Biology and Ecotoxicology of Large Marine Vertebrates: Potential Sentinels of Good Environmental Status of Marine Environment, Implication on European Marine Strategy Framework Directive. 31 January, Siena.
de Lucia, G.A., Caliani, I., Marra, S., Camedda, A., Coppa, S., Alcaro, L., Campani, T., Giannetti, M., Coppola, D., Cicero, A.M., Panti, C., Baini, M., Guerranti, C., Marsili, L., Massaro, G., Fossi, M.C., Matiddi, M., 2014. Amount and distribution of neustonic micro-plastic off the western Sardinian coast (Central-Western Mediterranean Sea). Mar. Environ. Res. 100, 10–16. http://dx.doi.org/10.1016/j.marenvres.2014.03.017.
de Stephanis, R., Giménez, J., Carpinelli, E., Gutierrez-Exposito, C., Cañadas, A., 2013. As main meal for sperm whales: plastics debris. Mar. Pollut. Bull. 69, 206–214.
Derraik, J.G.B., 2002. The pollution of the marine environment by plastic debris: a review. Mar. Pollut. Bull. 44, 842–852. http://dx.doi.org/10.1016/S0025-326X(02)00220-5.
European Commission, 2008. Directive 2008/56/EC of the European Parliament and of the Council of 17 June 2008, establishing a framework for community action in the field of marine environmental policy (marine strategy framework directive). Off. J. Eur. Union L164, 19–40.
European Commission, 2010. COMMISSION DECISION 2010/477/EU on criteria and methodological standards on good environmental status of marine waters. Off. J. Eur. Union L232, 14–24.
Fossi, M.C., Casini, S., Caliani, I., Panti, C., Marsili, L., Viarengo, A., Giangreco, R., Notarbartolo di Sciara, G., Serena, F., Ouerghi, A., Depledge, M.H., 2012. The role of large marine vertebrates in the assessment of the quality of pelagic marine ecosystems. Mar. Environ. Res. 77, 156–158.
Foti, M., Giacopello, C., Bottari, T., Fisichella, V., Rinaldo, D., Mammina, C., 2009. Antibiotic resistance of Gram Negatives isolates from loggerhead sea turtles (Caretta caretta) in the central Mediterranean Sea. Mar. Pollut. Bull. 58, 1363–1366.
Frazier, J., 2005. The role of flagship species in interactions between people and the sea. Marit. Stud. (MAST) 3, 5–39.
Frick, M.G., Williams, K.L., Pierrard, L., 2001. Summertime foraging and feeding by immature loggerhead sea turtles (Caretta caretta) from Georgia. Chelonian Conserv. Biol. 4, 178–181.
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.
Fukuoka, T., Yamane, M., Kinoshita, C., Narazaki, T., Marshall, G.J., Abernathy, K.J., Miyazaki, N., Sato, K., 2016. The feeding habit of sea turtles influences their reaction to artificial marine debris. Sci. Rep. 6, 28015. http://dx.doi.org/10.1038/srep28015.
Gabrielides, G.P., Golik, A., Loizides, L., Marino, M.G., Bingel, F., Torregrossa, M.V., 1991. Man made garbage pollution on the Mediterranean coastline. Mar. Pollut. Bull. 23, 437–441.
Gabrielsen, P., Bosch, P., 2003. Environmental Indicators: Typology and Use in Reporting. EEA internal working paper. 20pp.
Galgani, F., Jaunet, S., Campillo, A., Guenegen, X., His, E., 1995. Distribution and abundance of debris on the continental shelf of the north-western Mediterranean sea. Mar. Pollut. Bull. 30 (11), 713–717.
Galgani, F., Leaute, J.P., Moguedet, P., Souplet, A., Verin, Y., Carpentier, A., Gouraguer, H., Latrouite, D., Andral, B., Cadiou, Y., Mahe, J.C., Poulard, J.C., Nerisson, P., 2000. Litter on the sea floor along European coasts. Mar. Pollut. Bull. 40 (6), 516–527.
Galgani, F., Fleet, D., van Franeker, J., Katsanevakis, S., Maes, T., Mouat, J., Oosterbaan, L., Poitou, I., Hanke, G., Thompson, R., Amato, E., Birkun, A., Janssen, C., 2010. Marine Strategy Framework Directive e Task Group 10 Report Marine Litter. JRC Scientific and Technical Reports.
Galgani, Francois, Hanke, Georg, Werner, S., De Vrees, L., 2013. Marine litter within the European marine strategy framework directive. ICES J. Mar. Sci. 70 (6), 1055–1064. http://dx.doi.org/10.1093/icesjms/fst122. http://archimer.ifremer.fr/doc/00155/26586/24792.pdf.
Golik, A., Gertner, Y., 1992. Litter on the Israeli coast. Mar. Environ. Res. 33, 1–15.
Gramentz, D., 1988. Involvement of loggerhead turtle with the plastic, metal and hydrocarbon pollution in the central Mediterranean. Mar. Pollut. Bull. 19, 11–13.
Hamann, M., Godfrey, M.H., Seminoff, J.A., Arthur, K., Barata, P.C.R., Bjorndal, K.A., Bolten, A.B., Broderick, A., Campbell, L.M., Carreras, C., Casale, P., Chaloupka, M., Chan, S.K.F., Coyne, M.S., Crowder, L.B., Diez, C.E., Dutton, P.H., Epperly, S.P., FitzSimmons, N.N., Formia, A., Girondot, M., Hays, G.C., Cheng, I.S., Kaska, Y., Lewison, R., Mortimer, J.A., Nichols, W.J., Reina, R.D., Shanker, K., Spotila, J.R., Tomas, J., Wallace, B.P., Work, T.M., Zbinden, J., Godley, B.J., 2010. Global research priorities for sea turtles: informing management and conservation in the 21st century. Endang. Species Res. 11, 245–269.
Hoarau, L., Ainley, L., Jean, C., Ciccione, S., 2014. Ingestion and defecation of marine debris by loggerhead sea turtles, Caretta caretta, fromby-catches in the South-West Indian Ocean. Mar. Pollut. Bull. 84, 90–96.
ISPRA, 2013. Linee guida per il recupero, soccorso, affidamento e gestione delle tartarughe marine ai fini della riabilitazione e manipolazione a scopi scientifici. ISPRA, ISBN 978-88-448-0608-8. Manuali e Linee Guida 89/2013.
IUCN, 2013. IUCN Red List of Threatened Species. Version 2013.1 (Accessed November 2013). www.iucnredlist.org/.
Keller, J.M., McClellan-Green, P.D., Kucklick, J.R., Keil, D.E., Peden-Adams, M.M., 2006. Effects of organochlorine contaminants on loggerhead sea turtle immunity: comparison of a correlative field study and in vitro exposure experiments. Environ. Health Perspect. 114, 70–76.
Kühn, S., Rebolledo, E.L.B., Van Franeker, J.A., 2015. Deleterious effects of litter on marine life. In: Bergmann, M., Gutow, L., Klages, M. (Eds.), Marine Anthropogenic Litter. Springer, pp. 75–116.
Kühn, S., van Werven, B., van Oyen, A., Meijboom, A., Bravo Rebolledo, E.L., van Franeker, J.A., 2016. The use of potassium hydroxide (KOH) solution as a suitable approach to isolate plastics ingested by marine organisms. Mar. Pollut. Bull. http://dx.doi.org/10.1016/j.marpolbul.2016.11.034.
Laist, D., 1987. Overview of the biological effects of lost and discarded plastic debris in the marine environment. Mar. Pollut. Bull. 18, 319–326.
Lazar, B., Gracan, R., 2011. Ingestion of marine debris by loggerhead sea turtle, Caretta caretta in the Adriatic Sea. Mar. Pollut. Bull. 62, 43–47.
Lazar, B., Gracan, R., Kati_c, J., Zavodnik, D., Jaklin, A., Tvrtkov_c, N., 2010. Loggerhead sea turtles (Caretta caretta) as bioturbators in neritic habitats: an insight through the analysis of benthic molluscs in the diet. Mar. Ecol. 32, 65–74.
Lebreton, L.-M., Greer, S., Borrero, J., 2012. Numerical modelling of floating debris in the world's oceans. Mar. Pollut. Bull. 64 (3), 653–661.
Lusher, A.L., McHugh, M., Thompson, R.C., 2013. Occurrence of microplastics in the gastrointestinal tract of pelagic and demersal fish from the English Channel. Mar. Pollut. Bull. 67, 94–99.
Margaritoulis, D., Argano, R., Baran, I., Bentivegna, F., Bradai, M.N., Caminas, J.A., Casale, P., De Metrio, G., Demetropoulos, A., Gerosa, G., Godley, B.J., Haddoud, D.A., Houghton, J., Laurent, L., Lazar, B., 2003. Loggerhead turtles in Mediterranean Sea: present knowledge and conservation perspectives. In: Bolten, A.B., Witherington, B.E. (Eds.), Loggerhead Sea Turtles. Smithsonian Institution Press, Washington, DC, pp. 175–198.
Matiddi, M., van Franeker, J.A., Sammarini, V., Travaglini, A., Alcaro, L., 2011. Monitoring litter by sea turtles: an experimental protocol in the Mediterranean. In: Proceedings of the 4th Mediterranean Conference on Sea Turtles. 7–10 November, Naples Italy.
Matiddi, M., de Lucia, A.G., Travaglini, A., Baini, M., Mancusi, C., Cocumelli, C., Camedda, A., Serena, F., Campani, T., Scholl, F., Tomassetti, P., Hochscheid, S., 2015. Loggerhead sea turtle (Caretta caretta L., 1758) as biological indicator for impact of marine litter in Mediterranean Sea. In: Proceedings of the 35th Annual Symposium on Sea Turtles Biology and Conservation. 19–24 April, Dalaman Turkey.
Mrosovsky, N., 1981. Plastic jellyfish. Mar. Turt. Newsl. 17, 5–7.
Mrosovsky, N., Ryan, G.D., James, A.C., 2009. Leatherback turtles: the menace of plastic. Mar. Pollut. Bull. 58, 287–289.
MSFD TG Marine Litter 2013, Georg Hanke, François Galgani, Werner, Stefanie, Oosterbaan, Lex, Nilsson, Per, Fleet, David, Kinsey, Susan, Thompson, Richard C., van Franeker, Jan, Vlachogianni, Thomais, Scoullos, Michael, Mira Veiga, Joana, Palatinus, Andreja, Matiddi, Marco, Maes, Thomas, Korpinen, Samuli, Budziak, Ania, Leslie, Heather, Gago, Jesus, Liebezeit, Gerd, 2013. Guidance on Monitoring of Marine Litter in European Seas. EUR 26113. Luxembourg (Luxembourg). Publications Office of the European Union. JRC83985. http://publications.jrc.ec.europa.eu/repository/handle/111111111/30681.
MSFD GES TSG Marine Litter 2011, Galgani, F., Piha, H., Hanke, G., Werner, S., Alcaro, L., Matiddi, M., Fleet, D., Kamizoulis, G., Maes, T., Osterbaan, L., Thompson, R., Van Franeker, J., Mouat, J., Meacle, M., Carroll, C., Detloff, K., Kinsey, S., Nilsson, P., Sheavly, S., Svärd, B., Veiga, J., Morison, S., Katsanevakis, S., Lopez-Lopez, L., Palatinus, A., Scoullos, M., De Vrees, L., Abaza, V., Belchior, C., Brooks, C., Budziak, A., Hagebro, C., Holdsworth, N., Rendell, J., Serrano López, A., Sobral, P., Velikova, V., Vlachogianni, T., Wenneker, B., 2011. Marine Litter : Technical Recommendations for the Implementation of MSFD Requirements.

EUR 25009 EN. Luxembourg (Luxembourg). Publications Office of the European Union. JRC67300. http://publications.jrc.ec.europa.eu/repository/handle/111111111/22826.
Narazaki, T., Sato, K., Abernathy, K.J., Marshall, G.J., Miyazaki, N., 2013. Loggerhead turtles (*Caretta caretta*) use vision to forage on gelatinous prey in mid-water. PLoS One 8, e66043.
Nelms, S.E., Duncan, E.M., Broderick, A.C., Galloway, T.S., Godfrey, M.H., Hamann, M., Lindeque, P.K., Brendan, G.J., 2015. Plastic and marine turtles: a review and call for research. ICES J. Mar. Sci. http://dx.doi.org/10.1093/icesjms/fsv165.
Nicholson, E., Mace, G.M., Armsworth, P.R., Atkinson, G., 2009. Priority research areas for ecosystem services ina changing world. J. Appl. Ecol. 46, 1139–1144.
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.
Plotkin, P.T., Amos, A.F., 1988. Entanglement in and ingestion of marine debris by sea turtles stranded along the South Texas coast. In: Schroeder, B.A. (Ed.), (Comp.), Proceedings of the Eighth Annual Workshop on Sea Turtle Biology and Conservation. NOAA Technical Memorandum NMFS-SEFC-214. Forth Fisher, South Carolina, pp. 79–82.
Plotkin, P.T., Wicksten, M.K., Amos, A.F., 1993. Feeding ecology of the loggerhead sea turtle Caretta caretta in the North-Western Gulf of Mexico. Mar. Biol. 115, 1–15.
RAC-SPA, 2004. Guidelines to Improve the Involvement of Marine Rescue Centres for Marine Turtles. RAC-SPA.
Santos, R.G., Andrades, R., Fardim, L.M., Silva Martins, A., 2016. Marine debris ingestion and Thayer's law- The importance of plastic color. Environ. Pollut. 214, 585–588.
Schofield, G., Hobson, V.J., Fossette, S., Lilley, M.K., Katselidis, K.A., Hays, G.C., 2010. Biodiversity Research: fidelity to foraging sites, consistency of migration routes and habitat modulation of home range by sea turtles. Divers. Distributions 16, 840–853.
Schuyler, Q., Hardesty, B.D., Wilcox, C., Townsend, K., 2014a. Global analysis of anthropogenic debris ingestion by sea turtles. Conserv. Biol. 28, 129–139. http://dx.doi.org/10.1111/cobi.12126.
Schuyler OA., Chris Wilcox C., Townsend K., Hardesty BD., and Marshall NJ., 2014b. Mistaken identity? Visual similarities of marine debris to natural prey items of sea turtles MC Ecology, 14:14. http://www.biomedcentral.com/1472-6785/14/14.
Spear LB., Ainley DG.. Ribic AC, 1995. Incidence of plastic in seabirds from the tropical pacific, 1984–1991: Relation with distribution of species, sex, age, season, year and body weight. 40(2), 123–146.
Suaria, G., Aliani, S., 2014. Floating debris in the mediterranean sea. Mar. Pollut. Bull. 86, 1–2, 15, 494–504.
Suaria, G., Avio, C.G., Mineo, A., Lattin, G.L., Magaldi, M.G., Belmonte, G., Moore, C.J., Regoli, F., Aliani, S., 2016. The Mediterranean Plastic Soup: synthetic polymers in Mediterranean surface waters. Sci. Rep. 6, 37551. http://dx.doi.org/10.1038/srep37551.
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.
Travaglini, A., Matiddi, M., Ciampa, M., Alcaro, L., Bentivegna, F., 2013. Marine litter in loggerhead sea turtles (Caretta caretta) from Central and Southern Italian waters: analysis from dead and alive turtles. In: Proceedings of the Biology and Ecotoxicology of Large Marine Vertebrates and Sea Birds: Potential Sentinels of Good Environmental Status of Marine Environment, Implication on European Marine Strategy Framework Directive. 5–6 June, Siena.
Tucker, A.D., 2010. Nest site fidelity and clutch frequency of loggerhead turtles are better elucidated by satellite telemetry than by nocturnal tagging efforts: implications for stock estimation. J. Exp. Mar. Biol. Ecol. 383, 48–55.
Ullmann, J., Stachowitsch, M., 2015. A critical review of the Mediterranean sea turtle rescue network: a web looking for a weaver. Nat. Conserv. 10, 45–69. http://dx.doi.org/10.3897/natureconservation.10.4890. http://natureconservation.pensoft.net.
Underwood, A.J., 1997. Experiments in Ecology. Cambridge University Press, Cambridge, 504 pp.
UNEP(DEPI)/MED, 2012. Adoption of the Strategic Framework for Marine Litter Management. Decision IG.20/10, Athens Greece.
UNEP(DEPI)/MED, 2014. Monitoring Guidance on Ecological Objective 10: Marine Litter Correspondence Group on Monitoring, Pollution and Litter. WG.394/6, Athens Greece.
UNEP(DEPI)/MED, 2015. 1st Report of the Informal Online Working Group on Marine Litter. Meeting of the Integrated Monitoring Correspondence Group. WG.411/Inf.10, Athens Greece.
UNEP(DEPI)/MED, 2016. Implementing the Marine Litter Regional Plan in the Mediterranean (Fishing for Litter Guidelines, Assessment Report, Baselines Values, and Reduction Targets). Decision IG.22/13, Athens Greece.
UNEP(DEPI)/MED, 2017. Science Policy Interface and Ecosystem Approach Coordination Group Joint Meeting on IMAP Scale of Assessment and QSR. WG.438/6. Athens Greece.
Valente, A.L., Marco, I., Parga, M.L., Lavin, S., Alegre, F., Cuenca, R., 2008. Ingesta passage and gastric emptying times in loggerhead sea turtles (Caretta caretta). Res. Vet. Sci. 84, 132–139.
van Franeker, J.A., 2004. Save the North Sea Fulmar-Litter-EcoQO Manual Part 1: Collection and Dissection Procedures. Wageningen, Alterra, Alterra-rapport 672 pp.
van Franeker, J.A., Meijboom, A., 2002. Alterra-rapport. Litter NSV, Marine Litter Monitoring by Northern Fulmar; a Pilot Study, vol. 401, 72 pp.
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., Olsen, B., Olsen, K.O., Pedersen, J., Stienen, Erik, W.M., Turner, D.M., 2011. Monitoring plastic ingestion by the northern fulmar Fulmarus glacialis in the North Sea. Environ. Pollut. 159, 2609–2615.
Varo-Cruz, N., Hawkes, L.A., Cejudo, D., López, P., Coyne, M.S., Godley, B.J., López-Jurado, L.F., 2013. Satellite tracking derived insights into migration and foraging strategies of male loggerhead turtles in the eastern Atlantic. J. Exp. Mar. Biol. Ecol. 443, 134–140.
Werner, S., Budziak, A., van Franeker, J., Galgani, F., Hanke, G., Maes, T., Matiddi, M., Nilsson, P., Oosterbaan, L., Priestland, E., Thompson, R., Veiga, J., Vlachogianni, T., 2016. Harm Caused by Marine Litter. MSFD GES TG Marine Litter - Thematic Report. http://dx.doi.org/10.2788/690366. JRC Technical report; EUR 28317 EN.
Witherington, B.E., 1994. Flotsam, jetsam, post-hatchling loggerheads, and the advecting surface smorgasbord. In: Bjorndal, K.A., Bolten, A.B., Jonson, D.A., Eliazar, P.J. (Eds.), (Comps.), Proceedings of the Fourteenth Annual Symposium on Sea Turtle Biology and Conservation, pp. 166–168. NOAA Technical Memorandum NMFS-SEFSC-351.