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Ingestion of marine litter by loggerhead sea turtles, *Caretta caretta*, in Portuguese continental waters

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ABSTRACT

The accumulation of litter in marine and coastal environments is a major threat to marine life. Data on marine litter in the gastrointestinal tract of stranded loggerhead turtles, *Caretta caretta*, found along the Portuguese continental coast was presented. Out of the 95 analysed loggerheads, litter was present in 56 individuals (59.0%) and most had less than 10 litter items (76.8%) and less than 5 g (dm) (96.8%). Plastic was the main litter category (frequency of occurrence = 56.8%), while sheet (45.3%) was the most relevant plastic sub-category. There was no influence of loggerhead stranding season, cause of stranding or size on the amount of litter ingested (mean number and dry mass of litter items per turtle). The high ingested litter occurrence frequency in this study supports the use of the loggerhead turtle as a suitable tool to monitor marine litter trends, as required by the European Marine Strategy Framework Directive.

1. Introduction

Marine litter, defined as any persistent manufactured or processed solid waste in the marine and coastal environments (Coe and Rogers, 1997; Galgani et al., 2010), is presently recognized as a worldwide problem and a major threat to marine ecosystems (Galgani et al., 2010; Cole et al., 2011; Depledge et al., 2013; Pham et al., 2014). Globally, around 6.4 million tons of litter was estimated to reach the oceans annually, originated from both ocean and land-based sources (UNEP, 2005, 2009). However, further data on sources, inputs, degradation processes and fluxes are necessary to accurately estimate the global quantities of marine litter (Galgani et al., 2015). On average, three quarters of all marine litter consist of plastics, which are known to be particularly persistent in the environment (e.g. Galgani et al., 2010; Depledge et al., 2013; Pham et al., 2014).

The negative effects and threats of marine litter to marine life are primarily mechanical due to ingestion and entanglement in plastic packing straps, discarded fishing gear and other floating litter objects (Laist, 1997; Derraik, 2002; Katsanevakis, 2008). Entanglement and ingestion of marine litter affect hundreds of marine species worldwide including seabirds, fishes, sea turtles and marine mammals (Laist, 1997; Gall and Thompson, 2015; Kühn et al., 2015). Entanglement is a lethal and sub-lethal mortality factor since entangled animals may die from drowning, suffocation or strangulation and restricted movements and swimming, which in turn may hamper feeding and predator avoidance strategies (Laist, 1997; Derraik, 2002; Katsanevakis, 2008; Gall and Thompson, 2015; Kühn et al., 2015). Ingestion of marine litter by marine species cannot be readily detected and therefore it is under-reported when compared to entanglement (Gall and Thompson, 2015). Sub-lethal effects from litter ingestion include starvation due to gut obstruction and reduced fitness with consequences to reproduction and survival (Bjorndal et al., 1994; McCauley and Bjorndal, 1999; Derraik, 2002; Gall and Thompson, 2015; Kühn et al., 2015).

With respect to sea turtles, all seven species have been reported to ingest litter (Katsanevakis, 2008; Gall and Thompson, 2015; Kühn et al., 2015). Sea turtles may ingest large quantities of plastic litter that can be mistaken for food (Campani et al., 2013; Schuyler et al., 2014). These plastic fragments and other marine litter may be directly responsible for the obstruction of their digestive tracts and death (Bjorndal et al., 1994; Bugoni et al., 2001; Lazar and Gračan, 2011). Also, even at low ingestion rates marine litter is reported to have sub-lethal effects on sea turtles such as dietary dilution with consequent nutrient absorption reduction (McCauley and Bjorndal, 1999), and toxin uptake (Bjorndal, 1997) affecting growth rates, fecundity and survival.

The loggerhead sea turtle, *Caretta caretta*, is an Endangered species (IUCN, 2015). Litter ingestion by loggerhead turtles has been documented throughout the world (e.g., Boyle and Limpus, 2008; Frick et al.,

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2009; Camedda et al., 2014; Hoarau et al., 2014). Loggerheads are particularly susceptible to marine litter ingestion (Lutcavage et al., 1997) since marine litter can be mistaken for food mainly because of their foraging strategy, which is characterized by a high opportunistic behaviour (Lutcavage et al., 1997; Hoarau et al., 2014).

Loggerheads are one of the most common sea turtles occurring in Portuguese continental waters, especially in the southern region, which is an important development area for neritic juveniles (Nicolau et al., in press), originating mainly from western Atlantic rookeries travelling along the Gulf Stream and entering the Mediterranean through the Strait of Gibraltar (Carreras et al., 2006; Monzón-Argüello et al., 2009; Bellido et al., 2010).

Evaluating and understanding the impacts of anthropogenic pollution on marine organisms in the Atlantic Ocean are necessary to adequately address conservation issues. There is one study containing information about marine litter ingestion by loggerhead sea turtles in the Azores (Frick et al., 2009), but no information is available concerning litter ingestion by sea turtles in Portuguese continental waters.

The present study was performed according to the European MSFD (Marine Strategy Framework Directive) protocol for monitoring litter ingested by loggerhead turtles (MSFD GES Technical Subgroup on Marine Litter, 2011). Using loggerhead turtles stranded along the Portuguese continental coast, we aimed to assess the threat that marine litter represents to sea turtles in this region, and explore the possible influence of stranding season, cause of stranding and size of loggerhead turtles on litter ingestion.

2. Materials and methods

2.1. Study area

The Portuguese continental coast is 860 km long ranging from Caminha (41°50′N, 8°50′W) to Vila Real St. António (37°12′N, 7°25′W) (Fig. 1). The western coast (located between Caminha and Cape S. Vicente) diverges from the southern coast (located between Cape S. Vicente and Vila Real St. António) mainly due to their different topographic and oceanographic characteristics. Also northerly and northwesterly winds prevail in the western coast, whereas westerly and southern winds prevail on the southern coast (Fiúza, 1983).

2.2. Data collection

From August 2010 to September 2013, 95 stranded dead loggerhead sea turtles were collected along the Portuguese continental coast by the Portuguese Wildlife Society (SPVS) operating within the national marine animal stranding network (coordinated by the Institute of Nature Conservation and Forests, ICNF). Turtles presented a mean curved carapace length (CCL) of 49.8 ± 9.3 cm (mean ± SD) ranging between 25.4 and 75.5 cm. Detailed necropsies were performed and the likely cause of stranding was determined based on external and internal examination (Wyneken, 2001).

During necropsies, the entire digestive tract, from the beginning of the oesophagus to the end of the large intestine, was dissected from the animals. After its removal, the digestive tract was frozen or immediately sieved. Each digestive tract was subdivided into three parts: oesophagus, stomach and intestine, and then cut open. The contents were carefully emptied into a 1 mm mesh sieve and rinsed with water, avoiding any mixture of each section's contents. Marine litter was separated from food remains. Both litter and food remains were preserved in alcohol (70%), and then oven dried (40 °C) for 24 h before analysis. Diet analysis will be dealt with elsewhere. Marine litter items found in each digestive tract section were subdivided in 3 main categories (Plastic, Rubbish–other than plastics and Pollutants–industrial/chemical waste) that include 14 different sub-categories, according to the "European MSFD (Marine Strategy Framework Directive) protocol for monitoring litter ingested by loggerhead turtles (MSFD GES Technical Subgroup on Marine Litter, 2011). Natural litter (e.g. feathers, natural woods) was also included as a category, since it can also cause physical damage similar to the anthropogenic litter.

To avoid overestimating the amount of ingested litter, only fragments longer than 0.5 cm were considered an item. We considered that litter smaller than 0.5 cm may be generated by fragmentation of larger items inside the turtle or in the case of plastics, fall into the microplastic category (Arthur et al., 2009), which is out of the scope of the present study. For each turtle, we registered the total number, dry mass and length of every litter category and sub-category. The dry mass of food remains and the dry mass of total digestive tract content were also registered. The length of each litter item was measured using a ruler and the mean length of litter items was also calculated for each turtle. Plastic items were classified into colour categories (white, transparent, black, blue, green, brown, red, orange yellow and multi-colours).

2.3. Data analysis

Overall, the importance of each marine litter category and sub-category was assessed using the frequency of occurrence (FO%) (i.e. percentage of investigated digestive tracts containing litter). The frequency of occurrence was calculated on the entire sample ($n = 95$) as recommended by Kühn et al. (2015). In addition, the number of marine litter items and the dry mass of litter content was also calculated for each individual.

We used a permutational analysis of variance (PERMANOVA, 9999 runs) based on the Bray–Curtis index of similarity to evaluate the differences in debris composition between turtles according to litter sub-categories and colour (see above). The number of litter items and their mass were transformed using log $(x + 1)$ in order to meet the assumption of homoscedastic variances. Analyses were carried out using the free software PAST v. 2.12 (Hammer et al., 2001). Permanova analyses on litter sub-categories and colour included the fixed factor "season" with four levels (quartile 1: January–March ($n = 3$); quartile 2: April–June ($n = 32$); quartile 3: July–September ($n = 18$); quartile 4: October–December ($n = 3$)) and the fixed factor "cause of stranding" with three levels: bycatch ($n = 38$), other causes ($n = 5$) and undetermined ($n = 13$). Both designs were implemented in order to detect if stranding season or cause of stranding led to differences in the ingested item number and dry mass or in the number and dry mass of plastic litter according to their colours. Then, all data was aggregated because no effects related with season or cause of stranding were detected (see Results).

Since data did not present a normal distribution (even after transformation), a non-parametric Analysis of Variance (Kruskal–Wallis) was used to test differences between the number of items and dry mass of the considered litter categories (Plastic, Rubbish–other than plastics, Pollutants–industrial/chemical waste, Natural litter) and plastic colours, followed by a Dunn's test.

Loggerhead size-related differences were evaluated by regression analysis using Spearman rank correlation to determine the possible relationship between CCL and number and dry mass of litter ingested. Additionally, differences in number and dry mass of marine litter items were evaluated by a Mann Whitney-U test. For this analysis, loggerheads were split into two groups according to CCL: small juveniles (CCL ≤ 40 cm), which are going through the transitional pelagic–neritic life stage, and neritic individuals (CCL > 40 cm), which predominantly feed on the sea floor (Casale et al., 2008; Lazar et al., 2008).

3. Results

Marine litter items were present in 56 (59.0%) of the 95 analysed loggerhead turtles. In total, 920 pieces of litter were found, corresponding to 127.82 g (dry mass, dm). The mean (±SD) number of litter pieces