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Biases and best approaches for assessing debris ingestion in sea turtles, with a case study in the Mediterranean

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ABSTRACT

In a sample of 567 loggerhead turtles (*Caretta caretta*) from the central Mediterranean, debris occurrence varied according to methods and turtle source, and was up to 80% in pelagic turtles. Frequencies of plastic types, size and color are also reported. These results and a critical review of 49 studies worldwide indicate that: (i) the detected occurrence of plastic (% turtles) is affected by several factors (e.g., necropsy/feces, ecological zone, type and date of finding, captivity period for feces collection), (ii) mixed dataset and opportunistic approaches provide results which are biased , not comparable, and ultimately of questionable value, (iii) only turtles assumed to have had a normal feeding behaviour at the time of capture or death should be considered, (iv) turtle foraging ecology and possible selectivity may undermine the use of turtles as indicator species for monitoring marine litter, as recently proposed for the Mediterranean.

1. Introduction

Marine debris resulting from human waste enters the seas at the rate of eight million tons per year and rapidly increasing (Jambeck et al., 2015; UNEP, 2009). This anthropogenic debris accumulates both at surface in convergence zones (Cózar et al., 2014; Lebreton et al., 2012) and at sea floor at any depth (Pham et al., 2014), and represents an increasing threat for the marine environment as a whole (Gregory, 2009; Moore, 2008). Interaction of anthropogenic debris with marine wildlife includes entanglement, ingestion and smothering and has been documented for an increasing number of marine species (ca. 700 so far, including invertebrates, fish, birds, reptiles, mammals) (Gall and Thompson, 2015; Kühn et al., 2015). However, the impact of debris on marine species is still not well quantified and described, and possible mitigation measures are still at an early stage (Vegter et al., 2014).

Sea turtles interact with anthropogenic debris through entanglement and ingestion, with an increasing number of documented cases and studies (Balazs, 1985; Mrosovsky et al., 2009; Nelms et al., 2015; Schuyler et al., 2014a). Although direct exploitation, degradation of nesting habitat and bycatch are recognized as the major threats for these animals (Lutcavage et al., 1997; Wallace et al., 2013), emerging and less understood threats like anthropogenic debris and climate change are considered as reason of concern and priority areas of investigation (Hamann et al., 2010). Debris ingestion has been documented in all sea turtle species and in all ocean basins, with an high variability of occurrence among different studies (Nelms et al., 2015; Schuyler et al., 2014a). Debris may be accidentally ingested if mixed with natural food (e.g., Di Beneditto and Awabdi, 2014), actively selected because similar to natural preys, like jellyfish (Hoarau et al., 2014; Schuyler et al., 2012; Schuyler et al., 2014b) or because encrusted by natural prey (Frick et al., 2009). Debris can obstruct, damage or cause inflammation of the digestive tract (Bjorndal et al., 1994; Di Beneditto and Awabdi, 2014; McCauley and Bjorndal, 1999; Vélez-Rubio et al., 2013), causing a reduced digestive capability and even death. Even a small quantity of ingested debris can be lethal, at least in the green turtle *Chelonia mydas* (e.g., Bjorndal et al., 1994; Bugoni et al., 2001; Santos et al., 2015). When not lethal, ingested debris might cause other problems like a floating syndrome or a reduced swimming capability, making the turtle more vulnerable to bycatch or collision with boats. Bjorndal et al. (1994) suggested that debris may also have sub-lethal effects, possibly through the release of potentially harmful chemicals (Teuten et al., 2009). So far, only one study reported on such sub-lethal effects, and specifically dietary dilution (McCauley and Bjorndal, 1999).

Within a population, turtles may have a different foraging ecology, depending on the oceanographic features, age or individual preferences (Bolten, 2003; Casale et al., 2008; Rees et al., 2010) and therefore they may be exposed to different levels and types of debris. Moreover, the degree of ingestion or permanence of debris may be affected by the health status and debris may accumulate differently in different parts of the digestive tract. All these factors may induce severe biases when assessing the occurrence of debris, due to the great variety of methods

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used, even in the same study. Animals may be collected as strandings, bycatch, directly captured or picked while floating adrift. Presence of debris in dead animals is detected through necropsy, however different parts of the digestive tract may be collected and examined (esophagus, stomach, intestine). Debris from live animals may be obtained through esophagus lavage or feces. These different methods may weaken the comparison of different studies for deriving meaningful conclusions (Nelms et al., 2015; Schuyler et al., 2014a). The usually small sample size of such studies is another limiting factor for analyses.

All this is even more problematic if sea turtles are meant to be used as indicator species for monitoring marine litter. For instance, the EU Marine Strategy Framework Directive (MSFD) includes "Trends in the amount and composition of litter ingested by marine animals" among its indicators (Commission's Decision 2010/477/EU) and sea turtles are among the taxa considered as indicator species in the Mediterranean (Galgani et al., 2014), in the same way the bird *Fulmar glacialis* is for the north European seas (van Franeker et al., 2011). To this aim protocols and guidelines have been developed (Galgani et al., 2014; Galgani et al., 2013; Matiddi et al., 2011) and also implemented (Camedda et al., 2014; Campani et al., 2013).

So far, nine studies reported gut or feces contents of sea turtles in the Mediterranean, in the western (Camedda et al., 2014; Campani et al., 2013; Revelles et al., 2007; Tomas et al., 2002), south-central (Casale et al., 2008; Gramentz, 1988; Russo et al., 2003), Adriatic (Lazar and Gračan, 2011) and eastern (Kaska et al., 2004) zones. All studies examined loggerhead turtles and one also green turtles. However, only five were specifically investigating debris and in some cases debris occurrence might have been underreported.

Through the analysis of the largest sample collected so far by a single study and a critical review of published studies worldwide, we aim to (i) investigate the possible effect of different methodological approaches to the observed debris occurrence, (ii) improve the previous estimates about debris ingestion by sea turtles in the central Mediterranean (Casale et al., 2008), (iii) provide recommendations on data collection and analysis in order to enable meaningful comparisons among different studies, and (iv) contribute to the protocols and guidelines of the MSFD.

2. Methods

2.1. Sample collection

In seven years (2005, 2008, 2009, 2011, 2012, 2014, 2015), the ingestion of anthropogenic debris was investigated on a sample of 567 loggerhead turtles (*Caretta caretta*) brought to the sea turtle rescue centre in Lampedusa island, Italy (Fig. 1). Curved Carapace Length (CCLn-t; Bolten, 1999) of 561 turtles was measured. Turtles were found in the waters around Lampedusa (n = 461) or in Sicily (n = 106) in a variety of circumstances: picked while floating at sea surface (n = 282), incidentally caught by pelagic longliners (n = 135), by trawlers (n = 118), by other fishing gears (n = 11), or stranded (n = 21). No exact information on the place of incidental capture is available, however longliners typically fish in open waters off the continental shelves, while trawlers in shallow waters on the shelves, so that turtles caught by these two gears probably frequented the oceanic and neritic zones respectively (Fig. 1).

Part of the turtles (n = 29) were found already dead or died the day of arrival at the centre and were eventually necropsized. The other 538 turtles were kept in captivity in separate tanks for a period of 1–514 days until they were released or died. The presence of feces, including anthropogenic debris, was checked daily and any material was collected manually by a 10x10cm net of 0.4 mm mesh. At each collection event, the net was carefully inspected and all debris was removed and stored in a specific plastic bag labeled with the collection event data, then the net was cleaned. The ingestion of anthropogenic debris in dead turtles was assessed through necropsy, during which the

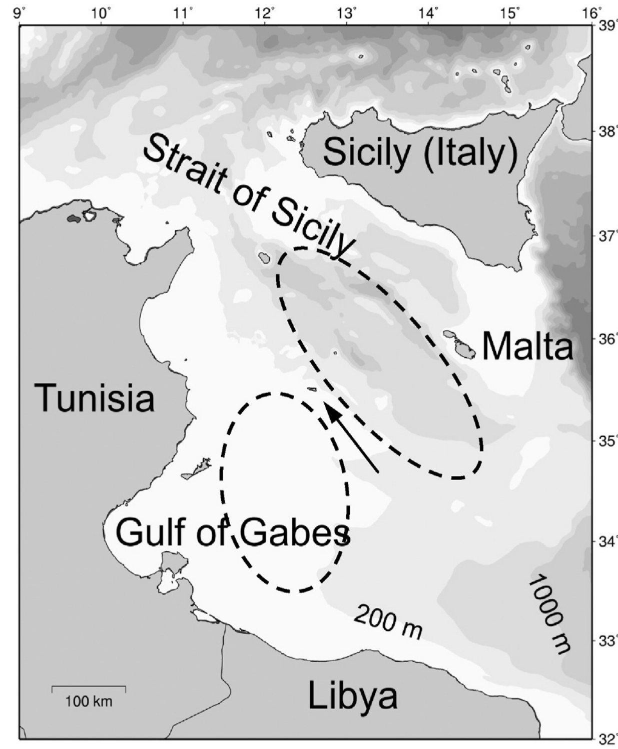

Fig. 1. Study area. The arrow indicates Lampedusa Island (Italy). Dashed lines show the approximate areas of fishing for trawlers (south) and longliners (north).

esophagus, stomach and intestine were carefully inspected and all debris was removed and stored in a specific plastic bag labeled with the necropsy data. The debris collected from feces or necropsy was stored at ambient temperature or frozen (−20 °C). During another phase, all material was rinsed with water multiple times and dried by means of both absorbent paper and air-drying.

The collected anthropogenic debris was subdivided into categories based on the OSPAR protocol developed for the bird *Fulmar glacialis* (van Franeker et al., 2011) and the guidelines for monitoring marine litter in the EU (Galgani et al., 2014; Galgani et al., 2013), and already implemented on Mediterranean loggerhead turtles (Camedda et al., 2014; Campani et al., 2013). Debris was also subdivided into 12 colour categories (orange, silver, white, dark blue, light blue, yellow, grey, brown, black, red, green, transparent). Debris pieces were measured (longest dimension) and weighted (0.1 g resolution).

2.2. Data analysis

Analyses and tests were performed by the programs R (R Development Core Team, 2015) and Excel. Confidence Intervals 95% of specific proportion of debris occurrence were estimated with the method for binomial distributions (Zar, 1999). Power analyses were performed through the the method for binomial distributions (Zar, 1999) and the pwr package for R (power = 0.9; h = 0.2 and 0.4, corresponding to 10% and 20% difference between two proportions around 0.5).

2.2.1. Debris occurrence

In order to investigate both linear and non-linear effects of categorical and continuous variables on the occurrence of debris on turtles, data were analyzed through generalized additive models (GAM) (Hastie and Tibshirani, 1986) performed using the gam function from the mgcv package for R. Specifically, models were in the form of D ~ TYPE + s(DATE) + s(DUR) + s(CCL), where D is the response variable (debris presence/absence) with a binomial distribution, TYPE is a categorical variable (type of finding, see above), and the