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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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A B S T R A C T

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

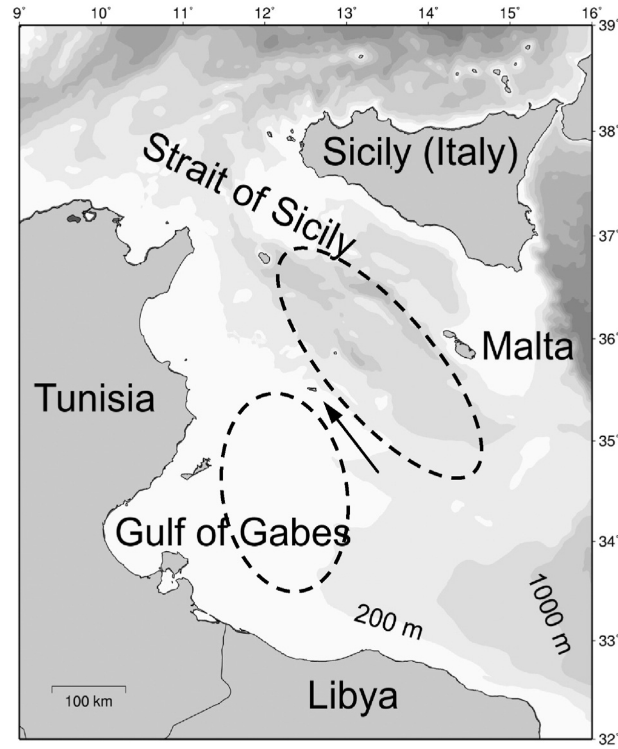

Fig. 1. Study area. The arrow indicates Lampedusa Island (Italy). Dashed lines show the approximate areas of fishing for trawlers (south) and longliners (north).

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 other three (DATE of finding; DUR: monitoring duration; CCL) are continuous predictor variables which affect the response variable via a 'smooth function' (s) estimated by non-parametric means. Five models were built. The first two models had the full set of smoothed variables as above, one with the original values of observation duration (1–514 days) and the second with right-censored values (23 turtles > 100 days were set at 100 days), based on the assumption that a time effect on the detection of debris occurrence was unlikely to occur beyond 100 days. The other three models had only two smoothed variables each. The fittest GAM was then compared to an analogous GLM (Generalized Linear Model; Nelder and Wedderburn, 1972) in order to ascertain the need of the additional complexity of GAM. GLM was performed using the glm function in R. The fittest model was selected on the basis of the lower AIC (Akaike's Information Criterion).

To test the hypothesis that strandings are more representative of coastal neritic turtles than of pelagic turtles as far as debris occurrence is concerned, stranded turtles were compared to turtles captured by trawl nets (fishing in neritic areas) and turtles captured by pelagic longline (fishing in oceanic areas) respectively.

2.2.2. Amount and type of debris

The possible effect of the type of finding on the debris abundance in term of number of pieces was investigated by a Kruskall-Wallis test performed by R (package pgirmess) on all the turtles with debris counts. In order to assess if the type of debris in terms of OSPAR and colour categories (see above) varied according to the type of finding (see above), two one-way PERMANOVA (permutational multivariate analysis of variance; Anderson, 2001) were performed using the adonis function from the vegan package for R (9999 permutations) on all the turtles with OSPAR and colour categories. In order to investigate possible association of categories and colours, a Fisher's exact test (with simulated P-value based on 10,000 MC replicates) was performed in R on a reduced 4 categories × 9 colours matrix obtained after removing low sample cases.

3. Results

Turtles ranged from 18.2 to 82 cm CCL (mean: 50.8; SD: 12.3; n = 561) (see Table S1 for complete CCL statistics by type of finding). Anthropogenic debris was observed in a total of 201 turtles out of 567. Necropsy of the 29 dead turtles found debris in five of them, and in one case plastic debris could be the cause of death, although the degree of decomposition made it impossible to ascertain it.

3.1. Timing of debris defecation

The timing of debris defecation was specifically investigated in a subsample of 47 turtles. They were monitored in captivity for a period of 1–250 days (median: 25; IQR: 14–42), during which the presence of debris in feces was observed from 1 to 5 times (median: 2; IQR: 1–2), with a period of 1–114 days (median: 4; IQR: 2–11) from their arrival to the first debris defecation and 1–129 days (median: 9; IQR: 4–16) from their arrival to the last debris defecation.

3.2. Debris occurrence

Debris occurrence did not significantly differ between turtles found around Lampedusa and in Sicily (Fisher's Exact Test, P = 0.43, n = 567), therefore they were considered as one group. Debris occurrence was significantly lower in those turtles found dead or which died the same day of finding (17.2%, n = 29) than in the other turtles which were kept in captivity for a variable period of time (36.4%, n = 538) (Fisher's Exact Test, P < 0.05, n = 567), therefore only the latter and larger group was considered for the following analyses about occurrence.

The GAM with three smoothed variables and right-censored duration (max 100 days) was considered to be the fittest model, since its AIC was lower than all the other GAMs and its analogous GLM. Its results are reported in Table 1 and show a significant effect of TYPE, DATE, and DUR on debris occurrence. While some TYPE categories had low sample sizes and consequently wide CI95%, the occurrence of debris in turtles captured by trawlers was lower than those captured by pelagic longliners or picked at sea surface (Fig. 2). The stranding category was significantly different from the pelagic longline category (Fisher's exact test, P < 0.05, n = 150) but not from the trawl category (Fisher's exact test, n = 131). The occurrence of debris ingestion varied along the 11-yr period of the study (DATE predictor), with recent years showing higher occurrence (Figs. 3, 4). The detection of debris ingestion was also greatly affected by the time the turtle was kept in observation (DUR predictor), with a simulated peak around 30 days (Fig. 5). However, this effect greatly varied among categories of finding (Fig. 6). A comparison between two arbitrary groups of approximately equal size, below and above 15 days in captivity, shows a significant effect of time on debris detection for the picked (Fisher's Exact Test; P < 0.05; n = 263) and especially for the longline category (Fisher's Exact Test; P < 0.001; n = 133) but not in the others. Therefore, for the picked and longline categories we considered the maximum values observed during long monitored periods (>25 days; picked: 0.41, CI95%: 0.32–0.51; n = 104; longline: 0.79, CI95%: 0.63–0.9; n = 38) to be closer to the real occurrence of debris, while for the other categories we considered the values obtained from the whole sample (trawl: 0.13, CI95%: 0.08–0.21, n = 114; strand: 0.24, CI95%: 0.07–0.50, n = 17). Debris occurrence per size class is shown in Fig. 7, with different apparent patterns in different finding categories.

3.3. Amount and type of debris

The amount of debris, in terms of number of pieces per turtle, ranged from 1 to 170 (median: 6; IQR: 2–12; n = 172) and was not significantly different among types of finding (Kruskall-Wallis test; H = 9.88; P = 0.08; n = 172). A total of 1820 debris particles were observed from 172 turtles, and were ascribed to four categories and nine subcategories (Table 2). The most common categories, both in terms of occurrence and number of particles, were SHE (remains of plastic sheets) and FRAG (fragments of thicker type plastics). No significant effect of the type of finding on the categories was detected (PERMANOVA, F = 1.41, P = 0.08). A total of 1775 debris particles from 171 turtles were ascribed to a colour category (Table 3), with white and transparent being the most common colours, both in terms of occurrence and number of particles. No significant effect of the type of finding on the colour was detected (PERMANOVA, F = 1.17, P = 0.24). Debris categories and colours were strongly associated (Fisher's exact test, P < 0.001, n = 1736). Most (58.5%) of the particles were soft plastic, 19.7% hard and 15.9%

Table 1
GAM results for presence of ingested debris (dependent variable) in loggerhead turtles in the central Mediterranean. PICKED (predictor category with the largest sample) is set as reference level (intercept). See text for model description.

Parametric coefficients				
	Estimate	Std. error	z value	P-value
(Intercept)	−0.621	0.140	−4.432	0.000
TYPE-DLL	1.150	0.941	1.222	0.222
TYPE-NET	0.014	0.901	0.016	0.988
TYPE-PLL	0.645	0.235	2.742	0.006
TYPE-STRAND	−0.929	0.630	−1.475	0.140
TYPE-TRAWL	−1.113	0.331	−3.361	0.001
Approximate significance of smooth terms				
	edf	Ref.df	Chi.sq	P-value
s(DATE)	2.225	2.714	11.96	0.007
s(DUR)	2.873	3.532	17.34	0.001
s(CCL)	1.919	2.434	4.79	0.110

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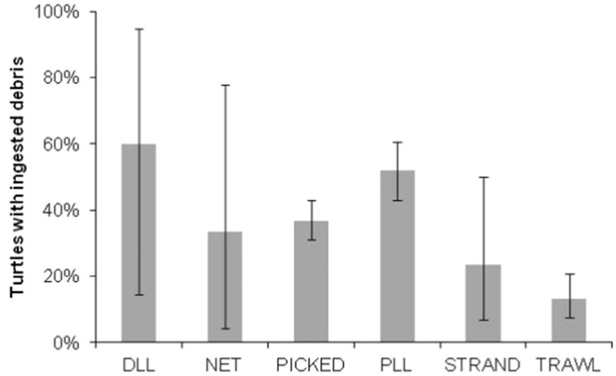

Fig. 2. Occurrence (% turtles) of ingested debris in loggerhead turtles in the central Mediterranean by type of finding: stranded (STRAND), picked at sea surface (PICKED), incidentally captures by demersal longline (DLL), pelagic longline (PLL), trawl net (TRAWL), net (NET) (n = 538). Vertical bars represent CI95%.

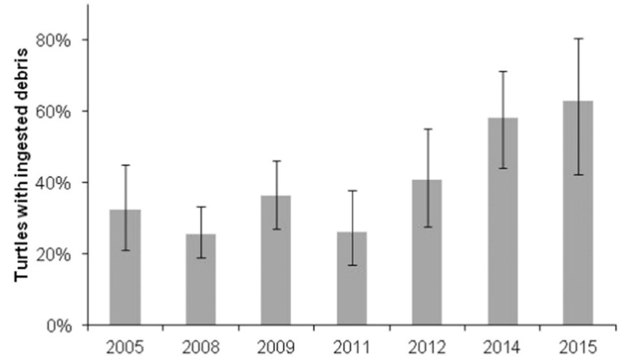

Fig. 4. Occurrence (% turtles) of ingested debris in loggerhead turtles in the central Mediterranean by year (n = 538). Vertical bars represent CI95%.

semi-hard plastic (n = 1775). Debris ranged from 0.26 to 53 cm (median: 2; IQR: 1.33–3.77; n = 1817) (Fig. 8). Total weight of debris per turtle (when at least 0.1 g) ranged from 0.1 to 9.6 g (median = 0.7; IQR: 0.30–0.38; n = 62), and the total weight was 82.5 g.

4. Discussion

This study analyzes the largest sample size collected so far by a single study on debris ingestion in sea turtles (Table 4). This made it possible not only to improve the current knowledge about debris ingestion by loggerhead sea turtles in the study area but also to provide indications on the methodological problems of such studies and possible solutions.

4.1. Debris ingestion by loggerhead sea turtles in the central Mediterranean

Results showed a very high occurrence (ca. 80%) of debris ingestion among turtles caught by pelagic longlines in contrast to those caught by trawl nets (13%). This represents a strong indication of both a different foraging behaviour by the two groups and a different distribution of debris. A previous diet analysis in the area (Casale et al., 2008) reported that turtles captured by pelagic longlines tend to feed on epipelagic prey, probably also because in those fishing areas the sea floor is often deep and benthic preys are less accessible, while turtles caught by bottom trawlers preferably feed on benthic prey. Such a difference in preferred preys or zones by the two groups suggests that turtles caught by pelagic longliners ingest more debris than those caught by trawlers because the epipelagic zone is where either most anthropogenic debris or debris resembling natural preys concentrates. Studies comparing debris ingested by turtles with debris at beaches and with turtle visual models suggest that other two sea turtle species (*Chelonia mydas* and *Eretmochelys imbricata*) selectively ingest anthropogenic debris with higher resemblance to their natural pelagic preys (Schuyler et al., 2012; Schuyler et al., 2014b). In the Mediterranean, anthropogenic debris sinking and accumulating at the sea floor at all depths do not seem to be less abundant than those floating (Deudero and Alomar, 2015; Pham et al., 2014; Ramirez-Llodra et al., 2013). We hypothesize that an active selection rather than availability is the best explanation for the observed higher occurrence of ingested debris in turtles caught by pelagic loglines than by trawl nets in our study. This is supported by the lack of difference in the type of ingested debris among turtle groups (finding categories) which indicates that all turtles select the same type of debris resembling the same type of prey. Specifically, the ingested debris was mostly composed of white or transparent plastic sheets or fragments, as also observed in other studies (e.g., Camedda et al., 2014; da Silva Mendes et al., 2015; Poli et al., 2015; Schuyler et al., 2012).

In the present study no significant relationship between debris occurrence and turtle size was observed, with just an apparent increase

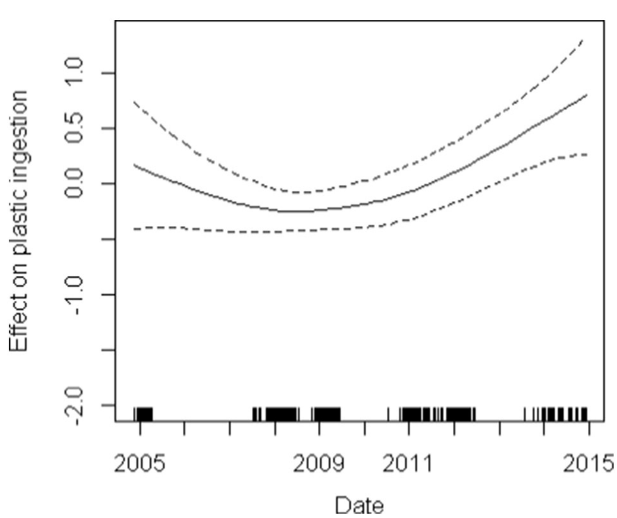

Fig. 3. Generalized additive model (GAM) derived effects of the time predictor DATE (date of finding, spanning years 2005–2015) on the presence of ingested debris in the loggerhead sea turtles in the central Mediterranean. The y-axis is the normalized effect of the variable; rugplot on the x-axis represents the observations; dashed lines represent ±2 SE.

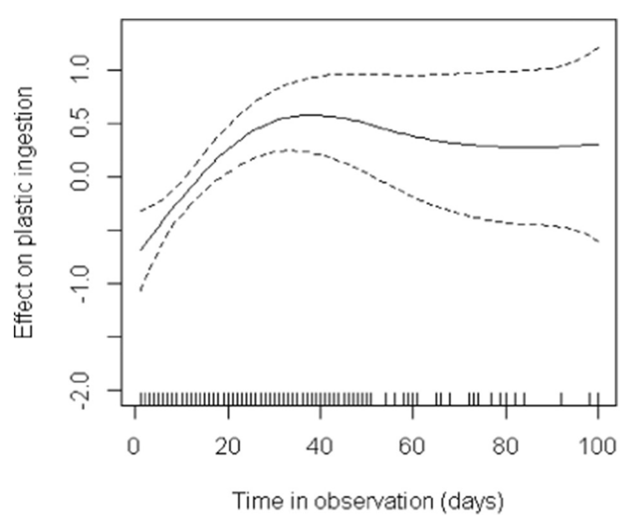

Fig. 5. Generalized additive model (GAM) derived effects of the time predictor DUR (duration of the monitoring of feces in captivity) on the presence of ingested debris in the loggerhead sea turtles in the central Mediterranean. The y-axis is the normalized effect of the variable; rugplot on the x-axis represents the observations; dashed lines represent ±2 SE.

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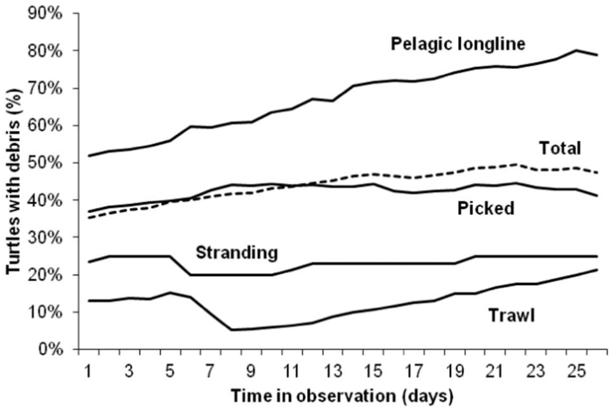

Fig. 6. Detected occurrence (% turtles) of ingested debris in the feces of loggerhead turtles in the central Mediterranean by the duration of the monitoring period, per type of finding. Time classes represent the minimum number of days, therefore a class includes individuals also included in all the upper classes. For classes 1 and 26, n total = 538 and 169 respectively.

of occurrence with size. This is probably because size is a poor predictor of loggerhead turtle foraging ecology in the Mediterranean, where different habitats are available at short distance and loggerheads prey on benthic animals whenever they can, starting from a small size (Casale et al., 2008). The possible increase of debris occurrence with size in our sample might depend on the longer digestive tract of larger animals, which would retain a piece of debris for longer than small animals, therefore increasing the chances that debris is detected. Floating material is more abundant in other areas like the western Mediterranean and the Adriatic sea than in the study area (Suaria and Aliani, 2014), therefore an even higher occurrence of debris ingestion is expected in loggerheads predominantly feeding upon pelagic preys in those areas. Both high and low levels of occurrence have been reported from the western Mediterranean (Table 4), however those studies did not attempt to identify a pelagic group within their sample.

In our sample, debris induced death in very few cases, if any. This mortality was not regularly reported by other studies (Schuyler et al., 2014a) and it seems to be more relevant for green (*Chelonia mydas*) and hawksbill turtles (*Eretmochelys imbricata*) (e.g., Bjorndal et al., 1994; Guebert-Bartholo et al., 2011; Poli et al., 2015; Santos et al., 2015). Mortality induced by debris was estimated at 10.7% over a large sample of green turtles in Brazil, where plastic amounts as low as 0.5 g caused death and with half of the observed deaths induced by <2.5 g of plastic (Santos et al., 2015). The amount of debris observed in our study in a subsample of turtles was maximum 9.6 g per individual. Nevertheless, the low mortality and low cases of blockage by debris

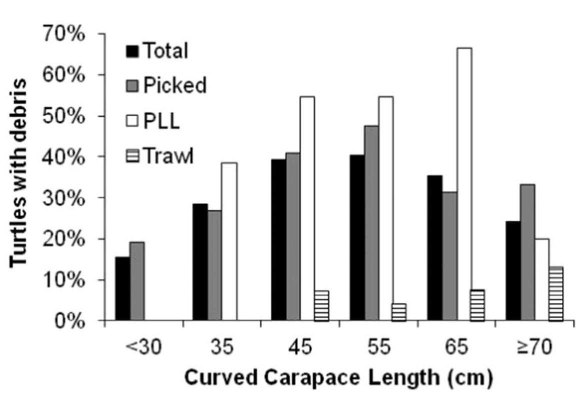

Fig. 7. Occurrence (% turtles) of ingested debris in loggerhead turtles in the central Mediterranean by size class and the three most abundant types of finding (n picked = 261; n PLL (pelagic longline) = 132; n Trawl = 81) and total (n = 535). Values on x-axis are the mid-point of the size class except for the first and last class.

Table 2
Type of debris ingested by loggerhead turtles in the central Mediterranean, by turtle and debris particle. Categories: POL (Pollutants); RUB (Other rubbish); PLA-IND (Industrial plastic); PLA-USE (User plastics). Sub-categories: TAR (oil/tar); RVA (other user); PAP: (paper); IND (pellets); THR (thread); POTH (other); SHE (sheet); FOAM (foam); FRAG (fragments). For more detailed descriptions see Galgani et al. (2013).

Categories		Turtles	%	Debris	%
POL	TAR	6	3.5%	7	0.4%
RUB	RVA	21	12.2%	36	2.0%
	PAP	3	1.7%	4	0.2%
	Total	24	14.0%	40	2.2%
PLA-IND	IND	3	1.7%	3	0.2%
PLA-USE	THR	44	25.6%	89	4.9%
	POTH	23	13.4%	33	1.8%
	SHE	141	82.0%	1041	57.2%
	FOAM	4	2.3%	25	1.4%
	FRAG	101	58.7%	582	32.0%
	Total	171	99.4%	1770	97.3%
Total		172	100.0%	1820	100.0%

reported by this and other studies (Lazar and Gračan, 2011; Plotkin et al., 1993; Tomas et al., 2002), suggests that loggerheads are less vulnerable to small quantities of debris than other species. In the reviewed studies which provided information on lethal cases (Table 4), the mortality rate among turtles with debris was significantly lower in loggerheads (2%; n = 146) than in green turtles (10%; n = 447) (Fisher's exact test; $P < 0.01$; n = 593). However, the very long permanence of debris in the turtle digestive tract (up to 129 days; IQR: 4–16 days), may increase the risk of sub-lethal effects like chemical contamination (Teuten et al., 2009).

4.2. Methodological problems of studies on debris ingestion by sea turtles

Present results showed that debris occurrence (% turtles with ingested debris) is greatly affected by several factors, in contrast to the characteristics of ingested debris (e.g., type, colour, etc). However, occurrence is usually the main target of studies aiming to assess or compare the impact of debris on sea turtles, and the primary goal of such studies may be undermined if those factors are not adequately considered.

4.2.1. Source and size of individuals

Thanks to a relatively large sample size, we could provide evidence that turtles from different sources are not statistically homogeneous in terms of debris occurrence. This is no surprise. Even within the same area and population, individual sea turtles may not represent an homogeneous group in terms of foraging ecology. Most species and populations have age phases or individual preferences for which individual turtles mainly feed on either pelagic or benthic prey (Bolten, 2003; Rees et al., 2010). Both the present and other studies (Schuyler et al.,

Table 3
Colour of debris ingested by loggerhead turtles in the central Mediterranean, by turtle and debris particle.

Colour	Turtles	%	Debris	%
Black	74	43.3%	155	8.7%
Blue	16	9.4%	27	1.5%
Brown	4	2.3%	4	0.2%
Green	21	12.3%	55	3.1%
Grey	8	4.7%	17	1.0%
Light-blue	25	14.6%	69	3.9%
Orange	1	0.6%	1	0.1%
Red	22	12.9%	35	2.0%
Silver	2	1.2%	2	0.1%
Transparent	111	64.9%	595	33.5%
White	126	73.7%	785	44.2%
Yellow	17	9.9%	30	1.7%
Total	171	100.0%	1775	100.0%

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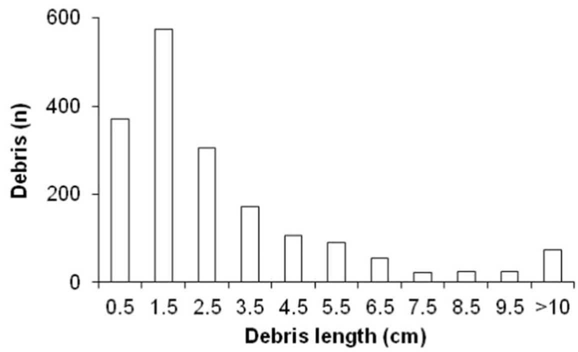

Fig. 8. Frequency distribution of debris particles (n = 1775) ingested by loggerhead turtles in the central Mediterranean per size class of the particle. Values on x-axis are the mid-point of the size class.

2015) provided evidence that turtles preferentially frequenting oceanic zones and feeding on pelagic prey ingest more debris, mainly plastics, than the others. Turtles incidentally caught in fishing gears can be ascribed to different groups if fishing gears can be linked to a specific habitat, like for bottom trawlers and pelagic longliners. Unfortunately, among the previous studies (Table 4) only a minority of turtles examined for debris occurrence were clearly ascribed to bycatch (22%) and of these only 57% were ascribed to a specific fishing gear. Moreover, in those cases with multiple fishing gears, usually debris occurrence was not provided for each fishing gear separately.

Stranded turtles represent by far the most used source of samples for these studies. However, even what is considered a stranding is not always clear. Stranding networks tend to pool together, explicitly or implicitly, animals found on the beach and those picked while floating at sea surface, assuming that a floating animal will eventually strand on the beach and that the two types are homogenous. This assumption proved to be wrong in a case study in the Mediterranean, probably for a bias towards live animals when rescuing animals at sea (Casale et al., 2010). In the present study, a difference of debris occurrence was observed between turtles stranded and bycaught by pelagic longliners but not between stranded and bycaught by trawlers. Although the lack of significance may be due to a small sample size, this finding is compatible with the hypothesis that stranded turtles are more representative of coastal/neritic turtles than of pelagic turtles, which would be coherent with the different distance a floating carcass should cover before washing ashore. Differently, Schuyler et al. (2015) did not find a significant difference between debris occurrence in stranded and bycaught turtles and concluded that strandings are representative of the normal population. We disagree with this conclusion. That lack of evidence could be due to the small sample size, possible confounding factors and the simplifications required by that global analysis, and finally a lack of evidence is not a proof of absence. In our local scale study we did observe a difference. We theorize that debris occurrence in stranding turtles may be biased towards either higher or lower levels, as a consequence of illness or injury which modify the normal foraging behaviour. Higher amount of debris may be observed if debris was the cause or concause of the stranding or it may be the result of an impediment to dive, forcing the turtle to feed at the sea surface only, where encountering floating debris is more likely. Lower amount of debris may be the consequence of a prolonged reduced feeding, including debris, and evacuation through feces of the already ingested debris. For instance Burke et al. (1994) reported empty digestive tracts in cold-stunned kemp's ridley turtles (*Lepidochelys kempii*). For these reasons, only turtles (stranded or not) which die almost immediately while healthy and presumably while feeding normally should be considered as representative of normal debris ingestion and included in such studies. With few exceptions (Macedo et al., 2011; Shaver, 1998), in most studies (Table 4) this aspect was not considered, and turtles suffering from cold-stunning, from other illness, entangled, or stranded because of debris ingestion were included in the analyses. In some cases, the body condition was mentioned in relation to this problem, but then all turtles were considered altogether in the analysis of occurrence (e.g., Guebert-Bartholo et al., 2011; Poli et al., 2015).

Although not observed in this study, probably for the reasons explained above, in some areas turtle size may be a proxy for the foraging ecology. Therefore, stratification per size classes may be informative and help comparisons with other studies.

4.2.2. Methods and focus

Studies where debris ingestion was not the primary goal (diet, mortality factors) reported a significantly lower occurrence of debris (25% and 7%, respectively) than those primarily focused on debris (55%) (Fisher's exact test; $P < 0.001$; n = 2881, 1673, respectively) (Table 4). This indicates a different quality of methods and data reporting and these aspects should be carefully considered by analytical reviews (e.g., Nelms et al., 2015; Schuyler et al., 2014a). For instance, fecal contents, examination of only parts of the digestive tract, and lower detection/reporting of debris are more common in studies not primarily focused on debris (Table 4).

While necropsy and examination of the digestive tract is the best way to ascertain the presence of ingested debris, debris is not equally distributed and accumulates mainly in the intestine (Bjorndal et al., 1994; Camedda et al., 2014; Campani et al., 2013; González Carman et al., 2014; Guebert-Bartholo et al., 2011; Macedo et al., 2011; Tomas et al., 2002; Tourinho et al., 2010). Therefore, examination of the esophagus and stomach only can easily underestimate the occurrence of debris, as initially suggested by Bjorndal et al. (1994).

Present results show that debris can be retained for long in the intestine and as a consequence the duration of the observation in captivity has an important effect on the detection of debris in feces. This means that studies using feces collected during relatively short periods (e.g., Burke et al., 1994) would likely underestimate debris occurrence. This is probably the main reason for the underestimation of debris ingestion obtained from feces, as highlighted by recent studies (Hoarau et al., 2014; Schuyler et al., 2014a).

4.2.3. Sample size and uncertainty

When assessing or comparing proportions, like the occurrence of ingested debris, sample size is crucial. Examining the ingested material of turtles, through feces or necropsy, is demanding for several reasons, first for the availability of turtles, and sample size is typically low. In previous studies sample size per study and species ranged from 1 to 265 turtles (median: 24; IQR: 9–54; n = 73; see Table 4 for samples ≥10, while the others are not listed). As a consequence, CI95% calculated for the debris occurrence reported by these studies are typically wide, making many occurrence values essentially meaningless (Table 4). For instance, a sample of 350 turtles would be needed to estimate a proportion of 35% with a 10% range of CI95% (i.e. 30–40%) and 90 turtles for a 20% range (25–45%). Regarding comparisons between two groups, 525 turtles for each group would be needed to detect a statistically significant difference of 10% (e.g., between 40 and 50%), while 131 turtles per group would be needed to detect a difference of 20% (e.g., between 40 and 60%). Nevertheless, a total of 161 pairwise statistical differences resulted among total debris occurrence within the three species where such a comparison was possible (loggerheads, green and kemp's ridley turtles) (Table S2). However, these differences should be considered with caution because they may be partially due to methodological differences (see above).

With the need of selecting representative animals and to stratify the sample, examining an adequate sample size for detecting small changes between areas or years becomes even more challenging. Even if the present study examined the largest sample collected so far by a single study, the sample size of the sub-groups resulting after adequate stratification is still too low to provide narrow CI95% and to detect a difference of 10% from another study.

Table 4
Debris ingestion in sea turtles reported by studied with N ≥ 10, per area and species (CC: *Caretta caretta*; CM: *Chelonia mydas*; DC: *Dermochelys coriacea*; EI: *Eretmochelys imbricata*; LO: *Lepidochelys olivacea*; LK: *Lepidochelys kempii*). CI95% were calculated (not provided by the original studies). CCL: Curved carapace length (unless otherwise specified: SCL, Straight carapace length). GI: gastrointestinal tract, including esophagus (E), stomach (S) and intestine (I); W indicates the whole tract; S*: it may be the whole tract. Stranded: washed ashore; Picked: at sea surface; Predated: found in the gut of predators; Captured: live capture research program. PL: pelagic longline; DL: demersal longline; T: trawl net; DN: demersal net; PN: pelagic net; S: seine; TUR: turtle fishery. Problems for occurrence estimate, C: no stratification per body condition in turtles stranded / picked; D: no stratification or raw data per turtle source; F: duration of monitored defecation not given; L: no clear assessment of lethal cases or problems induced by ingested debris; N: unclear total sample size; n: unclear number of turtles with debris; P: only part of GI examined; S: no size factor; T: no time factor; U: suspected low detection (underestimate).
Source: 1: present study; 2: Casale et al. (2008); 3: Russo et al. (2003); 4: Gramentz (1988); 5: Lazar and Gračan (2011); 6-7: Kaska et al. (2004); 8: Camedda et al. (2014); 9: Campani et al. (2013); 10: Tomas et al. (2002); 11: Revelles et al. (2007); 12: Duguy et al. (1998); 13: Duguy et al. (2000); 14: Frick et al. (2009); 15: Frick et al. (2001); 16: Burke et al. (1994); 17-18-19: Sadove and S.J. (1990); 20: Seney and Musick (2007); 21: Witherington (1994); 22: Foley et al. (2007); 23: Bjorndal et al. (1994); 24: Plotkin and Amos (1990); Plotkin et al. (1993); 25: Plotkin and Amos (1988, 1990); 26: Shaver (1991); 27: Shaver (1998); 28-29: Cannon (1998); 30-31: Duronslet et al. (1991); 32-33: Bugoni et al. (2001); 34: Santos et al. (2011); 35: Santos et al. (2015); 36: da Silva Mendes et al. (2015); 37: Awabdi et al. (2012); Di Beneditto and Awabdi (2014); 38: Tourinho et al. (2010); 39-40: Poli et al. (2014); Poli et al. (2015); 41: Guebert-Bartholo et al. (2011); 42: Ormedilla et al. (2014); 43: Vélez-Rubio et al. (2013); 44-45: Macedo et al. (2011); 46: González Carman et al. (2014); 47-48: Boyle and Limpus (2008); 49: Schuyler et al. (2012); 50: Limpus et al. (2001); 51: Parker et al. (2005); 52: Parker et al. (2011); 53-54: Wedemeyer-Strombel et al. (2015); 55: Peckham et al. (2011); 56: Lopez-Mendilaharsu et al. (2005); 57: Seminoff et al. (2002); 58: Quiñones et al. (2010); 59: Hoarau et al. (2014); 60: Hasbun et al. (2000).

	Area/focus	Species	Size CCL (cm)	Debris occurrence Total sample % (CI95%; N)	Debris occurrence GI/feces samples % (CI95%; N)	Stranded	Picked	Other (unknown, predated, captured)	Fishing	Lethal cases	GI part examined	GI part with debris	Problems for occurrence estimate
	Mediterranean Sea Central (Italy)												
1	Debris	CC		35.4 (31.5–39.5; 567)	GI: 17.2 (5.8–35.8; 29); Feces: 36.4 (32.4–40.7; 538)	21	282		264 (135 PL, 118 T, 6 DN, 5 DL)	1?	W	W	C
2	Diet	CC	25–80.3	48.1 (36.7–59.6; 79)	GI: 51.5 (33.5–69.2; 33); Feces: 45.7 (30.9–61; 46)	1	16	1 U	61 (26 PL, 35 T)	1	W	na	C, F, L, S, T
3	Mortality factors	CC	na	15.9 (6.6–30.1; 44)	All GI	?	?		?	0	W	I	C, L, S, T
	Central (Malta)												
4	Pollution	CC	20–69.5	≥8.1 (≤99)	GI: ≥7.1 (≤99); Feces: ≥1.0 (≤99)				≤99 PL	0	W	I	F, N, S, T
	Adriatic (Croatia, Slovenia)												
5	Debris	CC	25–79.2	35.2 (22.7–49.4; 54)	All GI	4		4 U	46	0	W	SI	C, D, T
	East (Turkey)												
6	Pollution	CC	47–80	≥2.4 (≤42)	All GI	≤42				na	na	na	C, L, N, n, U
7	Pollution	CM	30–84	0? (≤23)	All GI	≤23				na	na	na	C, L, N, n, U
	West (Italy)												
8	Debris	CC	21–73	14 (8.4–21.5; 121)	GI: 20 (7.7–38.6; 30); Feces: 12.1 (6.2–20.6; 91)	44	77			na	W	SI	C, D, F, L, T
9	Debris	CC	29–73	71 (52–85.8; 31)	All GI	?			?	na	W	SI	C, D, L
	West (Spain)												
10	Debris	CC	34–69	79.6 (66.5–89.4; 54)	All GI				54 T	0	W	SI	
11	Diet	CC	na	37.5 (19)	All GI	?	?		? DN	na	W	na	C, D, L, n, S, T
	Atlantic Ocean North-east (France)												
12	Mortality factors	CC		5 (0.1–24.9; 20)	All GI	?			?	na	na	S*	C, D, L
13	Debris	DC	na	55.2 (44.1–65.9; 87)	All GI	87				na	na	S*	C, L
	North-east (Azores, Portugal)												
14	Diet	CC	9.3–56	18.8 (4–45.6; 16)	GI: 25 (5.5–57.2; 12); Feces: 0 (4)	3	5		8 (7 PL)	0	S	S	C, F, P
	North-west (USA)												
15	Diet	CC	59.4–77	0 (12)	All GI	12					W		U
16	Diet	LK	24.7–42.7 SCL	3.4 (0.4–11.7; 59)	GI: 0 (40); Feces: 10.5 (1.3–33.1; 19)	40	2?		17		W		U
17	Debris	CC	na	8.6 (1.8–23.1; 35)	All GI	35				na	na	na	C, L, S, T

(continued on next page)

Table 4 (*continued*)

	Area/focus	Species	Size CCL (cm)	Debris occurrence Total sample % (CI95%; N)	Debris occurrence GI/feces samples % (CI95%; N)	Stranded	Picked	Other (unknown, predated, captured)	Fishing	Lethal cases	GI part examined	GI part with debris	Problems for occurrence estimate
18	Debris	LK	na	0 (44)	All GI	44				na	na	na	C, L, S, T
19	Debris	DC	na	30.3 (15.6–48.7; 33)	All GI	33				na	na	na	C, L, S, T
20	Diet	CC	41.6–98.5 SCL	0.6 (0–3.3; 166)	GI: ?; Feces: ?	?			?	na	W	na	C, D, F, L
21	Diet	CC	4.03–5.63	34 (21.2–48.8; 50)	All GI						ES	ES	P
22	Diet	CM	36.6	2.3 (0.1–12; 44)	All GI	?	?			na	W	na	L
23	Debris	CM	20.6–42.7	55.8 (39.9–70.9; 43)	All GI	43				2	W	W	C, T
Gulf of Mexico (USA)													
24	Diet	CC	51–105	51.2 (39.9–62.4; 82)	All GI	82				2	W	W	C, S, T
25	Debris	CM	na	46.7 (21.3–73.4; 15)	All GI	15					W	na	C, S, T
26	Diet	LK	5.2–71	28.7 (20.1–38.6; 101)	All GI	101					W	na	C, S, T
27	Diet	LK	na	18.9 (8–35.2; 37)	All GI	37				0	W	na	S
28	Mortality factors	LK	na	5.4 (2.5–10; 167)	All GI	167				0	W	na	C, S
29	Mortality factors	CC	na	5 (0.1–24.9; 20)	All GI	20				0	W	na	C, S
30	Debris	LK	na	45.5 (16.7–76.6; 11)	All GI	11				0	na		C, S
31	Debris	CC	na	68.4 (43.4–87.4; 19)	All GI	19				0	na		C, S
South-west (Brazil)													
32	Debris	CC	63–97	10 (0.3–44.5; 10)	All GI	10				na	ES	na	C, P, S
33	Debris	CM	28–50	60.5 (43.4–76; 38)	All GI	38				4	ES	na	C, P, S
34	Diet	CM	35.1–60	26.7 (7.8–55.1; 15)	All GI	15				na	S	S	C, P, S
35	Debris	CM	26.1–78.4	70.2 (64.3–75.6; 265)	All GI	265				20 ca.	W	na	C, n, S, T
36	Debris	CM	33–44	45 (23.1–68.5; 20)	All GI	20				2?	S	S	C, P
37	Debris	CM	na	59.2 (44.2–73; 49)	All GI			49 U		0	W	na	C, D, S
38	Debris	CM	31.5–56	100 (34)	All GI	34				3	W	W	C
39	Debris	CM	24–123.5	15.5 (8.5–25; 84)	All GI	84				9	W	SI	C, S
40	Debris	EI	48.8–21.6	41.7 (15.2–72.3; 12)	All GI	12				4	W	SI	C, S
41	Diet	CM	29–73	66.3 (54.8–76.4; 80)	All GI	71			9	4	W	W	C, D, S
42	Debris	CM	na	47 with debris	All GI	na				na	W	na	C, N, S

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Table 4 (*continued*)

	Area/focus	Species	Size CCL (cm)	Debris occurrence Total sample % (CI95%; N)	Debris occurrence GI/feces samples % (CI95%; N)	Stranded	Picked	Other (unknown, predated, captured)	Fishing	Lethal cases	GI part examined	GI part with debris	Problems for occurrence estimate
43	Debris	CM	na	55.6 (38.1–72.1; 36)	All GI					0	W	W	D, S
44	Debris	EI		77.8 (40–97.2; 9)	All GI					0			
South-west (Argentina-Uruguay)													
45	Debris	CM	31.3–52.2	90.3 (80.1–96.4; 62)	All GI				62 DN	0	W	W	S
46	Mortality factors	CM	na	≥56 with debris	All GI	≥56				na	na	na	L, N, n
PACIFIC OCEAN													
South-west (Australia)													
47	Diet	CM	5.5–11.3	73.5 (55.6–87.1; 34)	All GI	34				na	W	na	L
48	Diet	CM	5.9–9.4	46.2 (19.2–74.9; 13)	All GI			13 P		na	W	na	L
49	Debris	Mixed	5.4–105.8	33.9 (25.3–43.3; 115)	All GI	64		51 U		na	W	na	C, D, T
50	Diet	CC		0? (53)	GI: 0? (47); Feces: 0? (6)						W		U
Central north (Hawaii, USA)													
51	Diet	CC	13.5–74	34.6 (22–49.1; 52)	All GI				52 PN		S	S	P
52	Diet	CM	30–70	70 (34.8–93.3; 10)	All GI				10 (6 PL, 4 PN)		W	na	D
	North Pacific												
53	Debris	LO	58.9	82.2 (67.9–92; 45)	All GI				45 PL		W or S	na	P, T
54	Debris	CM	44.3	90.9 (70.8–98.9; 22)	All GI				22 PL		W or S	na	P, T
North-east (Baja California, Mexico)													
55	Diet	CC	na	0 (88)	All GI	88					S		P
56	Diet	CM	47.7–87	0? (24)	All GI				24 T, SE		S		P, U
57	Diet	CM	46–96.6	6.7 (3.2–11.9; 150)	GI: 3.7 (1–9.2; 108); Feces: 19 (8.6–34.1; 42)	5		42C	2	na	E or S	ES	F, P, S
South-east (Peru)													
58	Diet	CM	na	41.7 (34.6–49; 192)	All GI				192 TUR	na	ES	na	D, L, P, S, T
INDIAN OCEAN													
South-west (Reunion)													
59	Debris	CC	68.7	51.4 (39.4–63.1; 74)	GI: 42.9 (21.8–66; 21); Feces: 54.7 (40.4–68.4; 53)				74 PL		W	na	F, T
North-west (UAE)													
60	Diet	CM	>89	0? (13)	All GI	13					S		U

5. Conclusions and recommendations

The occurrence of plastic debris among loggerhead turtles foraging in the epipelagic zone of the Mediterranean and possibly elsewhere is probably much higher than previously thought, and it is likely that every turtle ingests some debris several times during its life. The interaction of several biological and methodological factors limits a correct comprehension of the debris ingestion phenomenon in sea turtles (Table 5). Opportunistic data collected by studies focused on different topics are particularly problematic in this respect. Results are often presented in a cryptic way and a generally adopted standardization is lacking, as shown by the fact that different reviews may report different results from the same study.

Table 5
Pros, Cons and possible solution for different sampling and methodological option in studies on debris ingestion by sea turtles, with specific focus on occurrence.

	Pros	Cons	Solutions
Turtle source			
Stranded/picked	>N	Potential bias (±)	Dead: include only rapid death Alive: exclude all
		Unknown neritic/oceanic	None/partial (diet)
Fishery	No bias	<N	More effort
	Zone-specific gear (neritic/oceanic)		Analysis by gear
Live capture	No bias	<N	More effort
	Known zone (neritic/oceanic)	No necropsy	Feces
Method			
Necropsy	Full content	<N	More effort
Feces	>N	Potential bias (−)	Long captivity
Lavage	>N	Bias (−)	None

Strandings represented the main source for such studies, however they suffer of several potential bias and a careful sampling is needed to derive estimations of debris ingestion in the population. Only turtles with evidence of a rapid dead can be safely assumed to be representative of their population, while in other cases this assumption would be weaker. Studies using stranded turtles should adopt a stratification by body condition. Other sources are preferable to strandings, e.g. bycatch, where turtles can be assumed to have died rapidly while being in health conditions and having a normal feeding behaviour. Stranding samples could be more useful for comparing the relative importance of different anthropogenic causes of death (e.g., Casale et al., 2010), including debris ingestion. Considering the need of an adequate stratification, the sample size required for detecting spatial and temporal differences is very high and challenging.

All these factors make it particularly problematic to use sea turtles as indicator organisms of the pollution status of sea areas and its trends, e.g. as proposed by the EU Marine Strategy Framework Directive (Galgani et al., 2014), making these goals probably unrealistic at the current level of research effort. Two aspects should be considered. First, regarding debris occurrence, sea turtles belonging to different categories may be biased in different ways. The currently proposed approach of using strandings (Galgani et al., 2014) is particularly problematic, because without a rigorous stratification, stranded turtles are probably biased regarding debris occurrence and this bias can vary with area and time, making comparisons or trends unreliable. It would be better to target floating debris only, through turtles feeding on pelagic prey sampled as bycatch in pelagic fishing gears. Second, regarding types and characteristics of debris, the possible selection of debris by turtles can make samples from turtles being not representative in general. On the other hand, present results show that debris composition is not affected by turtle category, therefore, differently from occurrence, samples from different sources may be pooled with less problem for investigating the type of debris.

The negative consequences of debris ingestion are far from being understood. Lethal effects of obstruction of the digestive tract are relatively obvious, however this is still understudied and the negative effects at population level cannot be easily derived at the present state of knowledge. Sublethal effects (e.g. from released toxics) might affect a larger part of the populations, however so far they have been only hypothesized (Bjorndal et al., 1994), except one study about dietary dilution (McCauley and Bjorndal, 1999).

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.marpolbul.2016.06.057.

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