

Are we even close? Five years marine litter ingestion monitoring in loggerhead turtles along Italian coast reveals how far we are from the Good Environmental Status

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ABSTRACT

The loggerhead sea turtle *Caretta caretta* has been chosen as bioindicator to monitor the amount of litter ingested by marine animals within the European Marine Strategy Framework Directive and the Barcelona Regional Sea Convention. European Member States and Contracting Parties are committed to achieve the Good Environmental Status (GES), which is reached when the quantity of ingested litter does not adversely affect the health of the species concerned. Although the monitoring strategy has been outlined for more than a decade, to date no threshold values have been adopted to verify GES achievement. After five years of extensive monitoring along the Italian coasts, this study evaluates the suitability of five different GES scenarios and proposes a new threshold value (i.e., "there should be less than 33% of sea turtles having more than 0.05 g of ingested plastic in the GI") for its implementation in the European seas and the Mediterranean basin.

1. Introduction

The constant growth of the human population and the consequent difficulties in waste management in the last decades have determined an increase in the amount of discarded solid waste worldwide. Synthetic

material such as plastic is the most abundant type of marine litter, and it is found in all oceans and seas of the world, even in remote areas far from human activities and contamination sources (Jambeck et al., 2015; Pelamatti et al., 2021). Plastic materials are made to be very durable and when dispersed in the environment can persist for a long time almost

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unaltered (Rochman et al., 2013). More than 900 animal species have been reported to be affected by marine debris interactions worldwide (Kühn and van Franeker, 2020). The most frequently documented detrimental impacts of marine litter on marine fauna are ingestion and entanglement (Worm et al., 2017). The effects of marine litter ingestion are variable, ranging from direct mortality (e.g., gastrointestinal blockage) to sublethal effects (e.g., behavioral changes, reduced feeding, release of absorbed toxic compounds, etc.) (Werner et al., 2016). The Mediterranean Sea has been identified as one of the most polluted areas of the world (Suaria and Aliani, 2014). It is a semi-enclosed basin characterized by evaporation exceeding precipitation and river runoff, in which the difference in water level leads to a net inflow of Atlantic superficial waters through Gibraltar (Millot and Taupier-Letage, 2005; Soto-Navarro et al., 2015). This limited outflow of surface waters together with the densely populated coasts and the high pressures posed by anthropic activities (e.g., coastal urbanization, marine traffic, tourism, fishery, offshore energy activities, and industries) determines the massive accumulation of marine litter (Cózar et al., 2015; Simon-Sánchez et al., 2022; Suaria and Aliani, 2014). At the same time, the Mediterranean Sea represents a hotspot for marine diversity, hosting over 600 marine vertebrates that are potentially threatened by the high amount of marine litter in the environment (Compa et al., 2019; Deudero and Alomar, 2015).

To preserve biodiversity and ecosystem functioning, the Mediterranean countries have developed several strategies to reduce the impact of marine litter on marine species. The Convention for the Protection of the Mediterranean Sea Against Pollution (Barcelona Convention) (<https://wedocs.unep.org>) is the most important regional and trans-boundary instrument aiming at protecting and promoting the sustainable development of the Mediterranean marine and coastal environment. The Convention Land Base Source Protocol has the objective to take all appropriate measures to prevent, abate, and eliminate various pollutants, including marine litter (UNEP, 1980; www.unep.org/unepmap). On the same wavelength, marine litter has been tackled by the Marine Strategy Framework Directive (MSFD, 2008/56/EC), one of the most ambitious international marine protection legal frameworks adopted in 2008 by the European Union. The MSFD Descriptor 10 (D10) commits to European Member States the monitoring of marine litter and its impact on marine biota, to reach the Good Environmental Status (GES, “the environmental status of marine waters where these provide ecologically diverse and dynamic oceans and seas which are clean, healthy and productive”; 2008/56/EC). The specific criteria D10C3 implies monitoring the amount of litter and micro-litter ingested by marine animals to keep it at a level that does not adversely affect the health of the species concerned (Commission decision 2017/848/EU). Therefore, Member States shall: i) develop a common monitoring strategy; ii) establish a set of specific bioindicators for marine litter; iii) define threshold values (TVs) to verify GES achievement at regional or subregional level. The first monitoring program of marine litter ingestion has been developed within the OSPAR Regional Sea Convention (Convention for the Protection of the Marine Environment in the North-East Atlantic) validating the northern fulmar (*Fulmarus glacialis*) as a good target species for measuring the impact of plastic pollution on the marine environment (van Franeker et al., 2011, 2021). Successively, the loggerhead sea turtle (*Caretta caretta*) has been designated for monitoring marine litter ingestion at lower latitudes and within the Barcelona Regional Sea Convention (Matiddi et al., 2011, 2017, 2019).

C. caretta is the most abundant sea turtle species of the Mediterranean Sea, and the only one that reproduces along the Italian coastline (ISPRA, 2013). Loggerhead turtles are long-lived animals maturing at about 80 cm curved carapace length (CCL) when they are between 24 and 30 years old (Casale et al., 2011). They can be considered good indicators of the environmental status of the Mediterranean basin since they have a well-known abundance and distribution, feed exclusively at sea, and have a long lifespan (Nicolau et al., 2016; Casale et al., 2018). In addition to these basic requirements, *C. caretta* is reported as frequently

affected by the ingestion of marine litter (Tomas et al., 2002; Lazar et al., 2011; Nicolau et al., 2016; Domènech et al., 2019) and samples are easily available through time and space by collecting stranded or by-caught animals (Camedda et al., 2014, 2022; Mariani et al., 2023). For all these reasons, the gastrointestinal contents of loggerhead turtles have been regularly analyzed to measure temporal trends and spatial differences in marine litter ingestion in the Mediterranean Sea (Matiddi et al., 2017).

Although the monitoring strategy has been outlined for more than a decade (Matiddi et al., 2011; Galgani et al., 2013), to date no threshold values (TVs) have been adopted to verify GES achievement. To respond to this specific request from the European Commission (European Commission, 2022), five different theoretical proposals (GES scenarios; Matiddi et al., 2017, 2019; Darmon et al., 2021) have been formulated, for each of which a reliability verification is necessary to confirm their capacity to return an adequate GES definition. In principle, the GES definition and the relevant TV must be precautionary and ambitious, aiming to achieve a pristine or near-pristine condition of the marine environment (Werner et al., 2016). Since a zero-pollution status cannot be reached for marine litter (i.e., plastic litter is resistant to natural degradation processes), all the proposed GES scenarios provide TVs determined by considering the less impacted MSFD area as a prospective GES reference (Matiddi et al., 2017, 2019; Darmon et al., 2021). Beyond this common point, each of the following GES scenarios is conceptually different from the others, proposing TVs based on different units of measurement, such as the number or the mass of ingested litter, the quantity of ingested litter according to the size of the individual examined, or the ratio between litter and natural food found in the gastrointestinal tract (GI) of each specimen (Matiddi et al., 2017; Darmon et al., 2021). Details on the five GES scenarios and their relevant TVs are reported in Table 1. The present study analyzes data from five years of data collection on litter ingestion by loggerhead sea turtles from Italian coastal waters with the aim to: i) disclose the use of *C. caretta* as bio-indicator of marine litter ingestion from an ecological perspective; ii) evaluate the suitability of the five GES scenarios and their achievement in the different subregions; iii) determine the current position of Italy concerning the proposed TVs; iv) develop a new threshold value based on the collected data (6th GES scenario).

2. Materials and methods

2.1. Sampling and data collection

Samples were collected between 2017 and 2021 along approximately 5135 km of Italian coasts (equal to 65 % of the national coastal extension; Fig. 1). According to the European Guidelines included in Galgani et al. (2023), all the analyses were performed following Matiddi et al. (2019). Briefly, *C. caretta* individuals found dead or in bad health conditions along the monitored coasts were transported to authorized centers and, in the event of death, analyzed through necropsy. Stranded turtles mummified, with broken or incomplete GI, and with a completely

Table 1

Good Environmental Status proposals (GES scenarios) and relevant threshold values according to Matiddi et al. (2017) and Darmon et al. (2021).

No.	Definition and threshold values
1	There should be no >26 % of individuals having >2 pieces of ingested plastic litter
2	There should be no >26 % of individuals having >0.32 g of ingested plastic litter
3	There should be no >25 % of individuals having >0.035 pieces 10 cm ⁻¹ of ingested plastic litter
4	There should be no >25 % of individuals having >0.091 g 10 cm ⁻¹ of ingested plastic litter
5	There should be <30 % of sea turtles having more weight of plastic (in grams) than food in the GI



Fig. 1. Map of Italy showing the three Marine Strategy Framework Directive subregions (i.e., Western Mediterranean Sea, Central Mediterranean Sea, and Adriatic Sea) and the relevant monitored coasts.

empty GI, were excluded from the analysis (Matiddi et al., 2019; Galgani et al., 2023). Curved carapace length (CCL) was measured to the nearest 0.1 cm for every individual. The possible cause of death or disease was determined by expert judgement after a visual inspection of external and internal conditions. The content of each gastrointestinal tract was emptied onto a 1 mm mesh sieve and washed carefully with freshwater. Items retained by the sieve were collected and preserved in 70 % ethanol solution until further characterization. All the collected materials were dried for 24 h before being sorted under a stereomicroscope. Plastic litter items were weighed to the nearest 0.001 g and counted. Remains of the natural diet of animals (FOO) were also recorded, dried, and weighed. For animals that died at the rescue center, any marine litter expelled during hospitalization (e.g., in feces) was collected, counted, weighed, and added to the data derived from the necropsy (Matiddi et al., 2019).

2.2. Data reporting and analysis

Marine litter ingestion rates were evaluated in terms of frequency of occurrence ($FO = 100 \cdot \text{no. of individuals with ingested litter} \cdot \text{no. of individuals examined}^{-1}$), number of ingested litter items (litter count, Lc), and grams of ingested litter (litter mass, Lm) per individual. Differences among subregions were tested using generalized linear models (GLMs) setting as response variables: the occurrence of litter ingestion (error structure: binomial, link = logit), Lc (error structure: negative binomial, link = log), and Lm (error structure: Gamma, link = inverse). The relationship between ingested litter and CCL was investigated through a generalized linear mixed-effects model for the negative binomial family, setting subregion as random effect. Spearman's rank correlation test was performed to verify the association between litter mass and FOO.

All statistical analyses were performed with R 4.3.2 (R Core Team, 2023) and the packages MASS (Venables and Ripley, 2002), lme4 (Bates et al., 2015), and ggplot2 (Wickham, 2016). The significance level was set at $p < 0.05$ for all the analyses.

2.3. GES scenarios

Five different GES scenarios were considered, two proposed by Matiddi et al. (2017) (hereafter, GES scenarios no. 2 and 5, as reported in Table 1), and three recommended by the INDICIT consortium (hereafter no. 1, 3, and 4; Table 1; Darmon et al., 2021). TVs reported in Table 1 were defined for all the GES scenarios, by the INDICIT consortium using a dataset of 802 samples collected in the whole Mediterranean basin and part of the Eastern Atlantic considering the area with the lowest percentage of litter ingestion and quantities of ingested litter as reference for GES definition (Darmon et al., 2021). In detail, TVs of GES scenarios no. 1 and 2 were based on the frequency of specimens with more ingested litter of the arithmetic litter means (2 pieces and 0.32 g respectively; Table 1). GES scenarios no. 3 and 4 considered pieces or grams of litter per size of individual estimated through CCL ($0.035 \text{ pieces } 10 \text{ cm}^{-1}$ and $0.091 \text{ g } 10 \text{ cm}^{-1}$). GES scenario no. 5 compared the grams of food remains (FOO) versus grams of ingested plastic, considering this relationship as a proxy of animal health.

An additional TV (hereafter GES scenario no. 6) was also defined following the UNEP/MAP 2023 MED QSR approach (UNEP/MED WG.550/13; <https://wedocs.unep.org>) and the EU GES assessment method for beach litter (Van Loon et al., 2020), which recommend a TV equal to the 15th percentile of the total litter abundance, a statistical method commonly employed in environmental assessments when precise reference values from pristine areas are unavailable (Van Loon et al., 2020). The UNEP/MAP 2023 MED QSR (UNEP/MED WG.550/13; <https://wedocs.unep.org>) adopted the same approach for seafloor litter and floating micro-litter, looking at the possibility of using a single statistical methodology for defining TVs for all the monitored environmental compartments, as well as defining five status classes, namely: high (observed value: $x \leq 0.5 \cdot TV$), good ($0.5 \cdot TV < x \leq TV$), moderate ($TV < x \leq 2 \cdot TV$), poor ($2 \cdot TV < x \leq 5 \cdot TV$), and bad ($> 5 \cdot TV$). The “high” and “good” status classes identify GES achievement. In the present study, this method was applied to data on litter ingestion by the loggerhead sea turtle to understand its ability in providing an adequate TV for the mass of ingested litter. Since in the dataset used for this study the 15th percentile is equal to zero, only turtles with ingested plastic were considered to compute the relevant TV, while the entire sample was used for comparison. GES computation was performed only for subregions with the required minimum sample size of 50 specimens (Matiddi et al., 2017, 2019; Darmon et al., 2021).

3. Results

3.1. Collection and description of samples

Between 2017 and 2021, a total of 459 loggerhead turtles (CCL mean \pm sd = 55.3 ± 14.9 cm) were collected and analyzed within the three Italian MSFD subregions: 231 from the Western Mediterranean (CCL mean \pm sd = 56.1 ± 13.8), 204 from the Adriatic Sea (CCL mean \pm sd = 54.9 ± 16.3), and 24 from the Central Mediterranean (CCL mean \pm sd = 51.1 ± 12.5). Most of the individuals were found dead stranded on the shoreline (73.6 %) or died at the rescue center after the rescue (15.9 %), while a lower number of specimens were discovered floating at sea (5.0 %) or by-caught during fishing activities (5.5 %). Necropsies revealed that marine litter ingestion was the cause of death in 12 individuals, suffering from obstruction of the digestive tract due to the accumulation of marine litter in correspondence of the pyloric valve.

3.2. Marine litter ingestion

Ingestion of marine litter was detected in 63.4 % of examined GIs, with an average \pm se of 9.97 ± 1.13 no. of items per individual (15.72 \pm 1.70 items per individual considering only GIs with marine litter) and an average \pm se litter mass of 1.34 ± 0.29 g (2.11 ± 0.45 g considering only samples with ingested marine litter). Statistical analyses highlighted significant differences in marine litter ingestion rates among the three MSFD subregions. Data showed an East-to-West increase of marine litter ingestion (i.e., Adriatic Sea < Central Mediterranean/Ionian Sea < Western Mediterranean) both in terms of frequency of occurrence, number, and mass of ingested items (Table 2). Regression analysis showed no association between CCL and the number of ingested litter items (intercept: estimate \pm se = 1.86 ± 0.53 , $Z = 3.49$, p -value < 0.001; coefficient: estimate \pm se = 0.00 ± 0.09 , $Z = -0.01$, p -value = 0.99) and a negative correlation between the occurrence and number of ingested litter items and food remains (Spearman rho correlation = 0.53; p -value < 0.01).

3.3. Computation for GES scenarios

According to the GES scenarios, to comply with the minimum number of samples required ($N = 50$), GES calculations were computed only for the whole Italy ($N = 459$), and the subregions Adriatic Sea ($N = 204$), and Western Mediterranean Sea ($N = 231$), while the Central Mediterranean Sea was excluded due to the low number of specimens collected ($N = 24$).

At the national level, TVs were exceeded for all GES scenarios with a general increasing trend of ingested plastic (Fig. 2; Table 3). The Adriatic Sea exceeds the TVs only for scenario no. 3 (i.e., % of turtles having ingested >0.035 pieces 10 cm^{-1}) and the Western Mediterranean subregion exceeds the TVs set by all the considered GES scenarios (Fig. 2;

Table 2

Incidence of marine litter ingestion in loggerhead turtles (*Caretta caretta*) collected along Italian coasts in 2017–2021. Coefficients \pm se estimates, test statistics, and p -values of regression models performed to test for differences among three Marine Strategy Framework Directive subregions (i.e., Adriatic Sea, Central Mediterranean Sea, and Western Mediterranean Sea). a) Frequency of occurrence (FO) and summary of a binomial GLM with logit link function; b) Average \pm se no. of ingested litter items (Lc) and summary of a negative binomial GLM with inverse link function; c) Average \pm se grams of ingested litter (Lm) and summary of a gamma GLM with log link function. Significance codes: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

Subregion	FO/Lc/Lm	Estimates \pm se	Z/T value	p-value
a)				
Intercept (Adriatic Sea)	40.7	-0.38 ± 0.14		
Central Mediterranean Sea	75.0	1.48 ± 0.49	3.00	0.003**
Western Mediterranean Sea	82.3	1.91 ± 0.22	8.55	<0.001***
b)				
Intercept (Adriatic Sea)	2.11 ± 0.36	0.75 ± 0.13		
Central Mediterranean Sea	8.29 ± 3.59	1.37 ± 0.38	3.65	<0.001***
Western Mediterranean Sea	17.10 ± 2.09	2.09 ± 0.17	12.29	<0.001***
c)				
Intercept (Adriatic Sea)	0.66 ± 0.14	1.53 ± 0.48		
Central Mediterranean Sea	1.05 ± 0.23	-0.57 ± 0.80	-0.71	0.478
Western Mediterranean Sea	2.86 ± 0.68	-1.18 ± 0.48	-2.43	0.016*

Table 3).

To develop the 6th GES scenario, the UNEP/MAP QSR 2023 assessment methodology (UNEP/MED WG.550/13) applied to data on the mass of litter ingested by *C. caretta* defines a TV equal to 0.05 g of ingested litter. The comparison of sub-regional data against the TV results in their classification under five status classes, which is summarized in Table 4.

4. Discussion

The present work represents the most comprehensive data collection regarding litter ingestion in dead loggerhead turtles from Italy. In the last decade, efforts have been made at both national and international levels to develop harmonized and standardized methodologies for the collection of data regarding the impact of marine litter on sea turtles (Camedda et al., 2014; Domènech et al., 2019; Darmon et al., 2021; Galgani et al., 2013; Matiddi et al., 2011, 2017, 2019) and such increased effort allowed the establishment of a consistent monitoring plan that led to the data presented in this study.

4.1. Incidence of marine litter ingestion

The overall frequency of litter ingestion in loggerhead turtles from 2017 to 2021 (63 %) is in line with previous results obtained at Mediterranean level by Darmon et al. (2022; FO = 69 %) and from Greek waters by Digka et al. (2020; FO = 72 %). At the subregional level, the FO detected in the Adriatic Sea (41 %) is similar to the FO found in specimens collected in the neighboring Croatia (Lazar and Gračan, 2011; FO = 35 %). Similarly, the higher incidence of marine litter ingestion noticed in the Western Mediterranean Sea (82 %) is consistent with previously published data on litter ingestion in the same subregion provided by Matiddi et al. (2017; FO = 85%), and from the Spanish Mediterranean coasts (Tomas et al., 2002; Domenech et al., 2019; FOs ranging from 78.1 % to 80.0 %). Although loggerhead turtle is a migratory species (Bentivegna, 2002), this similarity in differences among the Mediterranean sub-regions suggests that *C. caretta* is a good bioindicator to provide a measure of marine litter bioavailability at the subregional scale. In contrast, care should be taken when interpreting the differences at smaller geographical scales, since loggerhead turtles can travel long distances and the transit time of ingested items is usually between 10 and 14 days (Polovina et al., 2003; Valente et al., 2008; Solomando et al., 2022).

The review by Moon et al. (2023) pointed out a negative correlation between marine litter ingestion and sea turtles' CCL, supposing that this correlation can be explained hypothesizing that younger sea turtles of smaller sizes may be more frequently exposed to marine litter ingestion. Domènech et al. (2019) and Wedemeyer-Strombel et al. (2015), indicated the opposite trend, while no correlation was found by Camedda et al. (2014), and Nicolau (2016). In the present study the lack of correlation between the CCL and the mass, or the number of plastic items ingested by sea turtles, can be explained by assuming that at any stage of the animal's development, the exposure to marine litter pollution remains the same along the Italian coast, as juvenile, subadult, and adult sea turtles occupy the same trophic niche, as mentioned by Mariani et al. (2023).

4.2. GES

The results show that none of the six GES scenarios has been reached in favor of the loggerhead turtles within the Italian waters. Respecting the request for a minimum of 50 samples, the Central Mediterranean Sea has been excluded from the computation of GES scenarios. This result highlights that an appropriate assessment could be difficult in some subregions, suggesting the need of more extended studies to optimize the efforts required to collect samples and obtain reliable data in terms of statistical power.

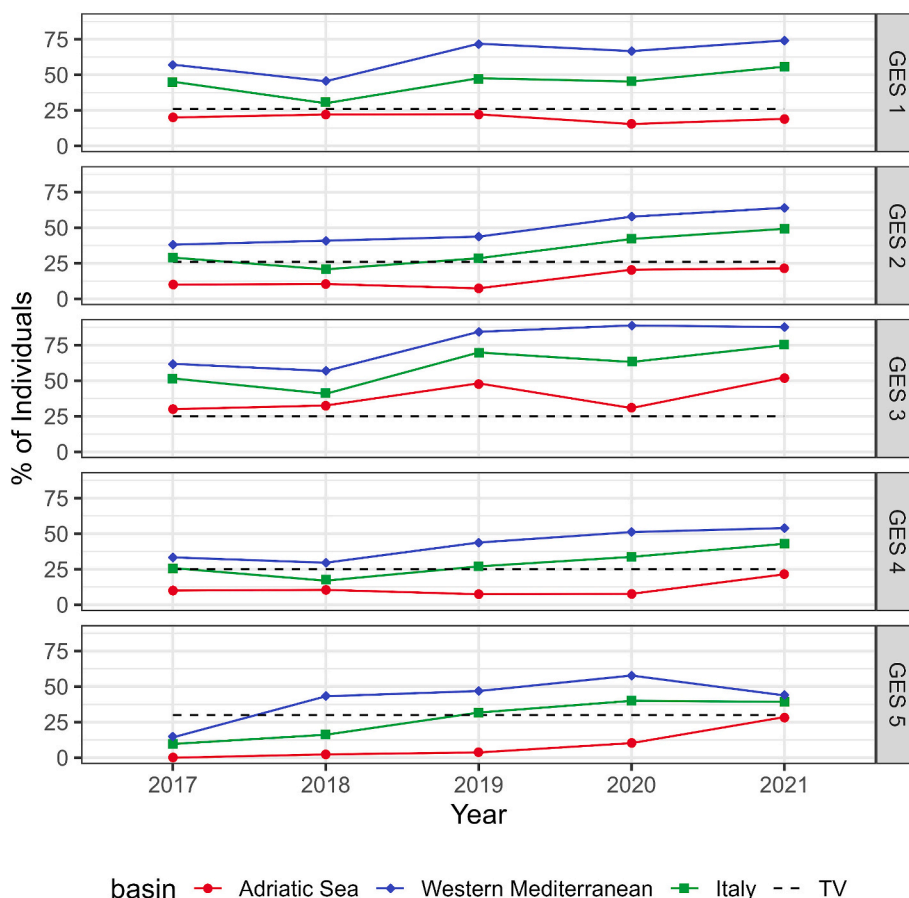


Fig. 2. Computation for five different GES scenarios applied to loggerhead sea turtles collected in Adriatic Sea (red circles), Western Mediterranean Sea (blue diamonds), and Italian coasts (green squares) from 2017 to 2021. GES 1: There should be no >26 % of individuals having >2 pieces of ingested plastic litter; GES 2: There should be no >26 % of individuals having >0.32 g of ingested plastic litter; GES 3: There should be no >25 % of individuals having >0.035 pieces 10 cm⁻¹ of ingested plastic litter; GES 4: There should be no >25 % of individuals having >0.091 g 10 cm⁻¹ of ingested plastic litter; GES 5: There should be <30 % of sea turtles having more weight of plastic (in grams) than food in the GI in samples of 50/100 dead turtles from each sub-region. TV: threshold values (dashed lines). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 3
Evaluation of Italian loggerhead turtles in the context of previously proposed GES scenarios, considering all Italian subregions, Adriatic Sea, and Western Mediterranean separately. Values above the threshold value (TV) set for each scenario are highlighted with *.

No.	Brief description	TV	All subregions	Adriatic Sea	Western Mediterranean Sea
1	% having ingested >2 pieces	26	46*	21	67*
2	% having ingested >0.32 g of plastic	26	36*	14	53*
3	% having ingested >0.035 pieces 10 cm ⁻¹	25	63*	41*	82*
4	% having ingested >0.091 g 10 cm ⁻¹	25	32*	12	49*
5	% of sea turtles having more grams of plastic than food in the GI	30	30	9	44*

4.2.1. *Caretta caretta* as bioindicator: Ecological implications

Considering the results from the different subregions analyzed, it appears that the Western Mediterranean Sea subregion is far from GES achievement for all the considered GES scenarios. On the other hand, the Adriatic Sea seems to be too cleaner than expected considering results

Table 4
Percentage of Adriatic Sea and Western Mediterranean turtles according to the GES/NO-GES classification adapted from UNEP/MED WG.550/13.

Boundary limits	Status classes	WM Turtles	AS Turtles	GES achievement
$x \leq 0.5 \bullet TV$	High	26 %	63 %	GES
$0.5 \bullet TV < x \leq 2 \bullet TV$	Good	4 %	4 %	NO-GES
$TV < x \leq 2 \bullet TV$	Moderate	5 %	4 %	
$2 \bullet TV < x \leq 5 \bullet TV$	Poor	7 %	10 %	
$> 5 \bullet TV$	Bad	57 %	18 %	

from litter assessment of other environmental compartments (e.g., beach and sea surface; Fortibuoni et al., 2021; Lambert et al., 2020). Indeed, it is interesting to note that beach litter abundance on the Italian coast does not reflect the results obtained considering ingestion by loggerhead turtles. The Adriatic Sea coastline is the most polluted subregion (590 items/100 m of beach), followed by Western Mediterranean Sea (491 items/100 m) and the Ionian Sea and Central Mediterranean Sea sub-region (274 items/100 m) (Fortibuoni et al., 2021). This difference might be related to the stranding process determined by the Adriatic Sea's elongated shape and the effects of dominant winds. During winter the bora blowing from north-east pushes floating litter toward the Italian Adriatic coast, where it is found after winter perturbations (Bertotti and Cavaleri, 2009). For this reason, loggerheads in their Adriatic

feeding ground are likely to be exposed to lower amounts of floating litter that, once discarded from the main freshwater input (*i.e.*, the Po River) is quickly pushed by northern winds toward the Italian Adriatic coast, where is not bioavailable to be ingested by marine organisms.

This eventuality is not confirmed by data from the international basin-scale survey of the Mediterranean Sea to provide abundance estimate of floating mega-debris (>30 cm) (Lambert et al., 2020). The authors reported the highest densities occurred along the Italian Tyrrhenian coast facing the Western Mediterranean Sea, and in the Adriatic Sea, with up to 20 items per km². This discrepancy with data on litter ingestion might depend on the abundant availability of food in the Adriatic Sea and the energy optimization strategy of sea turtles during their feeding. Our data show the high food remains abundance in samples from the Adriatic Sea (14,741 g; average 115,17 g) compared to the Western Mediterranean samples (2214 g; average 10,35 g). The Adriatic Sea represents the main Italian ground for aquaculture and mussels' farmers reaching around the 80 % of the National production (ISPRA, 2023). Since the loggerhead is an opportunistic animal, when abundant benthic prey like crustaceans or mollusks are available (as in the case of *Mytilus galloprovincialis* in the Adriatic Sea; Mariani et al., 2023), sea turtles may not be attracted by other resources, promoting a selective behavior that may lead to reduced plastic ingestion. Otherwise much simpler, sea turtles that can't find enough available food in the environment, feed any other items available, both natural and non-natural, including plastic. In this view, the huge availability of food in the Adriatic Sea affects the suitability of all the GES scenarios.

4.2.2. Suitability of GES scenarios

Commenting upon each scenario, number 1 and 2 seems to work well when there is not a huge displacement between food and litter. However, it is important to notice that when wide monitoring is assessed and different staff are employed, scenario number 1 can be biased by different methodologies of enumeration. Some operators could count the number of all the plastic fragments found in the digestive tract, whereas other operators could group the pieces which could maybe originate and have fragmented from the same item during turtle feeding (Matiddi et al., 2019). In this view, the mass represents a quantitative measure excepted from subjective bias and we strongly suggest the use of this unit of measurement applied in scenario number 2. In contrast, scenarios number 3 and 4 do not provide suitable tools for monitoring as we do not find an association between animals' size and ingested litter, and international data are controversial (*cf.* Camedda et al., 2014; Domenech et al., 2019; Moon et al., 2023).

Some additional considerations should be made on the proposed TVs and the compilation of a historical data series allows the understanding of the impact of marine litter on *C. caretta* and comparing observed data with previous theoretical formulation. For instance, it has been previously estimated that turtles with >14 pieces of plastic in their GI have a 50 % probability of mortality because of litter ingestion (Wilcox et al., 2018). We do not find a correlation with this formulation, as ingestion of debris has been identified as the direct cause of death only in 12 cases (2.6 % of the examined turtles), in which 50 % had <5 items, while 16.3 % of examined individuals had >14 items. However, it is still possible the eventuality of sub-lethal effects due to chronic exposure to plastic ingestion, like starvation and reduced feeding that, to a lesser extent, contributed to other death causes (Worm et al., 2017).

Nevertheless, TVs should provide no impact on animal health, while our data reveals that among 12 turtle death due to litter ingestion, around 17 % ingested <0.32 g (*i.e.*, the TV fixed by the 2nd GES scenario). The use of the 15th percentile, proposed in the 6th scenario, seems to be more appropriate and conservative, in respect to the average value and no lethal impact has been observed in individuals with <0.05 g of ingested litter. Following this consideration, according to our dataset elaboration, the most suitable GES scenario should be:

- There should be <33 % of sea turtles having >0.05 g of ingested plastic in the GI in sample of at least 50 dead turtles from each sub-region.

The availability of a big dataset, following wide monitoring, could better define the above TV and the relative percentage of affected turtles. The classification under 5 status classes, according to the UNEP/MAP method, could be very useful for policy programme of measures since it also allows tracking the distance from GES achievement and consistently driving local remediation actions.

One of the main limitations of the GES scenarios is the missing link between marine litter ingestion and the health of the species concerned, as requested by the new Commission Decision 848/17/EC in the criteria D10C3. It also happens in the GES scenario proposed for *F. glacialis* within the OSPAR countries (Van Franeker et al., 2021). The 5th GES scenario intends to address this issue by using the ratio between food remains and marine litter as a proxy for the health condition of the individuals. Despite it appears suitable, our data shows that the relationship between marine litter ingestion and feeding activities of *C. caretta* can be complex and influenced by the active behavior of loggerhead turtles during food selection. Indeed, though the Adriatic Sea is affected by a massive presence of marine litter (Lambert et al., 2020), the high abundance of food availability in this subregion ensure that loggerheads' nutrition is not strongly affected by marine litter ingestion.

Our results point out that *C. caretta* cannot be considered as a marine litter passive sampler and marine litter bioavailability results not only from its detectability but also from the active decision of the animals, which are in turn driven by the environmental context that they spent life stage and the habitat use. In this view, further research is needed to understand the impact of different types of marine litter, and to implement a GES scenario connecting marine litter ingestion to the health of the species, considering that scenarios 5 and 6 can serve as a good starting point.

5. Conclusion

In conclusion, to satisfy the D10C3 criteria of the MSFD as well as the UNEP/MAP Ecological Objective 24, sea turtles cannot be considered as a simple net to collect marine litter but monitoring results should be interpreted taking into consideration the ecology of the species and local environmental circumstances. In this view this criterion should be considered as an indicator of marine litter impact instead of pressure, as considered until now. Urgent measures are therefore needed to reduce the input of litter in the marine environment and international measures are necessary to achieve a decreasing trend that can get the MSFD Member States closer to reaching a Good Environmental Status in the near future. Education, monitoring and policy are essential for the conservation of *Caretta caretta* as well as the protection of the entire marine ecosystem.

CRedit authorship contribution statement

Marco Matiddi: Writing – original draft, Validation, Supervision, Resources, Project administration, Methodology, Conceptualization. **Tommaso Valente:** Writing – original draft, Validation, Formal analysis, Conceptualization. **Andrea Camedda:** Writing – review & editing, Investigation, Data curation. **Cinzia Centelleghé:** Writing – review & editing, Investigation, Data curation. **Cristiano Cocumelli:** Writing – review & editing, Investigation, Data curation. **Salvatore Dara:** Writing – review & editing, Investigation, Data curation. **Giuseppe Andrea de Lucia:** Writing – review & editing, Investigation, Data curation. **Ludovica Di Renzo:** Writing – review & editing, Investigation, Data curation. **Nicola Ferri:** Writing – review & editing, Investigation, Data curation. **Giorgia Gioacchini:** Writing – review & editing, Investigation, Data curation. **Sandra Hochscheid:** Writing – review & editing, Investigation, Data curation. **Giuseppe Lucifora:** Writing – review &

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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