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Marine turtles as bio-indicators of plastic pollution in the eastern Mediterranean

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ABSTRACT

The loggerhead turtle (*Caretta caretta*) has been suggested as a bio-indicator species for plastic pollution. However, detailed investigations in the eastern Mediterranean are limited. Here, we present data from loggerhead turtles (2012-2022; $n = 131$) of which 42.7 % ($n = 57$) had ingested macroplastic (pieces $\geq$ 5 mm). Frequency of occurrence (%) was not found to have changed over time, with body size (CCL cm), between stranded or bycaught turtles, or with levels of digesta present. The characteristics of ingested plastic ($n = 492$) were largely *sheetlike* (62 %), *clear* (41 %) or *white* (25 %) and the most common polymers identified were Polypropylene (37 %) and Polyethylene (35 %). Strong selectivity was displayed towards certain types, colours and shapes. Data are also presented for posthatchling turtles ($n = 4$), an understudied life stage. Much larger sample sizes will be needed for this species to be an effective bio-indicator, with the consideration of monitoring green turtles (*Chelonia mydas*) for the eastern Mediterranean recommended allowing a more holistic picture to be gathered.

1. Introduction

High densities of marine plastic pollution are now present across oceanic gyres, coastal waters and beaches (van Sebille et al., 2015), putting long-lasting pressure on marine systems (Barnes et al., 2009). An estimated 8 million metric tons of plastic enter the ocean each year (Jambeck et al., 2015), which is predicted to increase 2.6-fold by 2040 (Lau et al., 2020). The widespread dispersion and mobility of plastic pollution and its presence in all marine habitats allows it to interact with a wide variety of biota (Gall and Thompson, 2015). Plastic represents a threat to multiple marine vertebrate species through ingestion, entanglement and degradation of key habitats (Duncan et al., 2017; Fackelmann et al., 2023; Fuentes et al., 2023; Nelms et al., 2016, 2019; Schuyler et al., 2014a, 2014b; Wilcox et al., 2013), although population-scale impacts have been more difficult to determine (Senko et al., 2020).

Records of plastic ingestion in marine turtles are now ubiquitous across all species and ocean basins including; the Atlantic (Colferai et al., 2017; Di Beneditto and Awabdi, 2014; Eastman et al., 2020; Machovsky-Capuska et al., 2020; Mascarenhas et al., 2004; Pham et al., 2017a; Rice et al., 2021; Rizzi et al., 2019; Santos et al., 2016; Witherington, 2002), the Pacific (Clukey et al., 2017; Godoy and Stockin, 2018; Jung et al., 2018; Ng et al., 2016; Wedemeyer-Strombel et al., 2015), the Indian Ocean (Hoarau et al., 2014; Yaghmour et al., 2018, 2021), the western Mediterranean Sea (Bruno et al., 2022; Camedda et al., 2014, 2022a,

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2022b; Campani et al., 2013; Darmon et al., 2022; Digka et al., 2020; Matiddi et al., 2017; Solomando et al., 2022; Tomas et al., 2002) and the eastern Mediterranean sea (Duncan et al., 2019; Darmon et al., 2022). Ongoing, long-term monitoring is important to observe trends in ingestion, to understand potential future population level impacts related to this threat, and also track concentrations of plastic pollution in marine environments (Darmon et al., 2022; Senko et al., 2020). The loggerhead turtle (*Caretta caretta*) is one of the most studied marine species for this threat due to its widespread global distribution, with frequency of occurrence (FO%; proportion of turtles assessed that contained ingested plastic) being highly variable globally (0-90 %) (Lynch, 2018).

A major global plastic pollution hotspot is the Mediterranean Sea, where between 873 and 2576 t of plastic debris is estimated to be floating on the sea surface (Cózar et al., 2015; Suaria et al., 2016). The loggerhead turtle has been designated as a bio-indicator species under the EU Marine Strategy Framework Directive (MSFD) (2010) (2010/477/EU) (Galgani et al., 2014; Matiddi et al., 2017), for evaluating the Good Environmental Status (GES) at the Mediterranean and European scales (Darmon et al., 2022). It has also been proposed as a bio-indicator species for monitoring under The Convention for the Protection of the Marine Environment of the North-East Atlantic (OSPAR) and the Barcelona Convention for the Protection of the Marine Environment and the Coastal Region of the Mediterranean (Casale and Ceriani, 2020). However, despite inclusion within these conventions, records of ingestion in loggerhead turtles within the eastern Mediterranean basin are limited (Darmon et al., 2022). Therefore, collecting and reporting detailed information about the ingestion of plastic in this data poor region of the Mediterranean Sea is crucial for contributing to the efforts of monitoring GES (Galgani et al., 2014; Matiddi et al., 2017; Darmon et al., 2022).

Studies from the eastern Mediterranean have demonstrated the presence of widespread post-pelagic foraging grounds for loggerhead turtles around North Cyprus (Haywood et al., 2019; Palmer et al., 2021, 2024; Snape et al., 2013). Year-round strandings and bycatch occurs with seasonal peaks during the summer months, providing opportunities to directly assess gut content (Palmer et al., 2021; Snape et al., 2013). Here we sought to augment knowledge of plastic ingestion by loggerhead turtles in the eastern Mediterranean region by: 1. Assessing abundance and occurrence in loggerhead turtles while considering turtle size, whether animals were stranded or bycaught, and according to levels of digesta present; 2. Exploring temporal and spatial trends; 3. Describing plastic ingested according to type, colour, shape and polymer; 4. Investigating potential selectivity of plastic ingestion compared to environmental baseline levels of beach debris. All data are presented in a form to facilitate building extensive datasets and future meta-analyses.

2. Material & methods

2.1. Study area and sample collection

This study was conducted in North Cyprus, in the eastern Mediterranean basin. The island hosts important nesting beaches and foraging grounds for loggerhead turtles (Haywood et al., 2019; Omeyer et al., 2021). The coastline is regularly patrolled for nest monitoring and stranded turtles, as well as having fisheries-focused research and public awareness activities that facilitate the discovery, reporting, and transportation of stranded or bycaught turtles (fresh dead to moderately decomposed) to the project base for necropsy (with permission obtained from the North Cyprus Department of Environmental Protection, Animal Husbandry and Veterinary Department). The vast majority of turtles subjected to necropsy are considered to have died as a result of bycatch incidents in coastal small-scale fisheries, typically being drowned in bottom-set trammel nets (Snape et al., 2016).

A total of 135 loggerhead turtles were sampled spanning a period of 11 years (2012–2022; Fig. 1.). Given the size distribution of the turtles

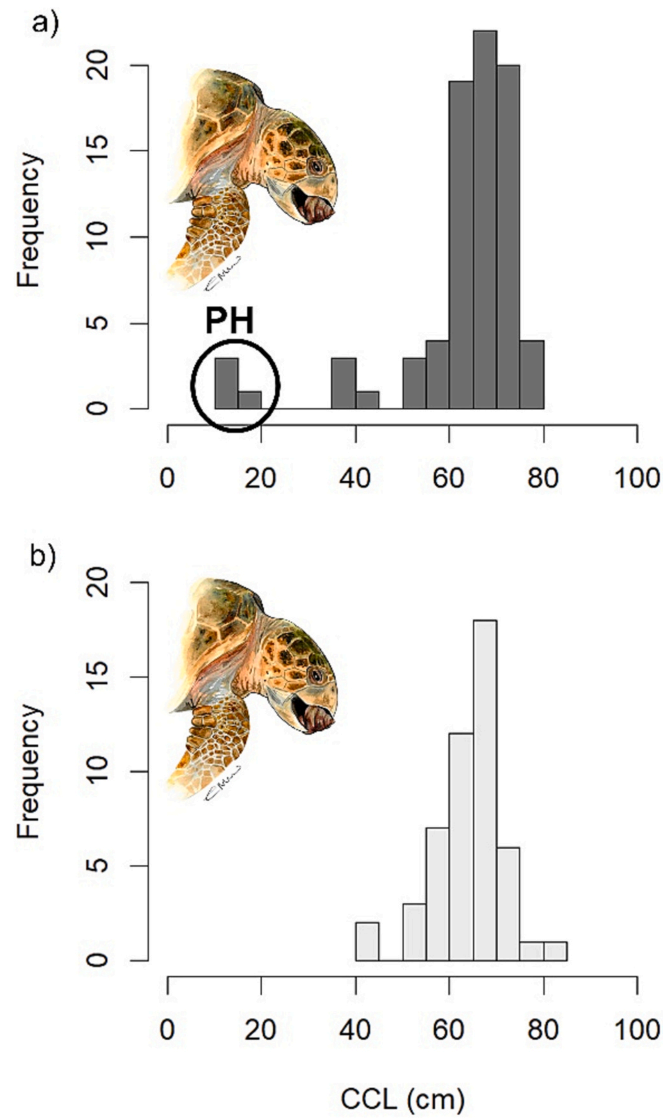

Fig. 1. Size of turtles investigated Frequency histogram of loggerhead turtles (*Caretta caretta*) according to curved carapace length (cm) where data were available for a) stranded turtles (dark grey; $n = 80$; $n = 4$ of which were posthatchlings: PH) and b) bycaught turtles (light grey; $n = 49$). For 6 individuals CCL measurements were not obtained. Original artwork by Emma Wood.

investigated, the majority ($n = 131$; 97 %) were post-pelagic juveniles and adults with CCL >35 cm (Casale et al., 2008). There was additional access to a small number of stranded posthatchling turtles ($n = 4$), relatively rare for the region (Levy et al., 2015; Türkozan et al., 2013), that were likely still in their pelagic life phase. These were considered separately and presented to facilitate future collaborative analyses. During necropsy, the entire gastrointestinal tract was removed and initial contents weighed. The contents were grossly examined then rinsed through a 1 mm mesh sieve to separate and remove the plastic for classification and dietary items for storage and analysis (Palmer et al., 2021). Turtles demonstrated varying levels of gut fill and were classified as those with normal levels of digesta (NLD) present therefore exhibiting recent feeding activity, i.e. digested and partially digested remains of multiple prey items, and those with a distinct lack of recognisable dietary items with low levels of digesta (LLD), i.e. no discernible food items present, containing only sediment and digestive fluids.

To establish if there was a difference in plastic ingestion between stranded turtles and those known to be freshly captured in fisheries or if varying levels of gut ingesta may correlate with differences in plastic incidence, we considered post-pelagic turtles in four groups separately in the first instance: Stranded NLD (n = 72), Stranded LLD (n = 9), Bycaught NLD (n = 45), Bycaught LLD (n = 5). Additionally, from previous studies, beach plastics around North Cyprus are known to vary spatially (Duncan et al., 2018; Özden et al., 2021) so turtles with from known stranding or bycaught locations were grouped according to the areas of the coast from where they were obtained; East (n = 68), North (n = 37), West (n = 13), Unknown (n = 14).

To normalise for turtle size the body burden of ingested plastic (g plastic/kg turtle) was calculated following calculations outlined in Clukey et al. (2017) and Lynch (2018). Body condition index (BCI) (turtle mass kg/SCL3) was also calculated following indices described in Bjorndal et al. (2000) and Rice et al. (2021) to compare with measures of plastic burdens.

2.2. Plastic classification

Ingested plastic was classified using a system outlined in Duncan et al. (2019, 2021) which builds upon the Fulmar Protocol and MSFD (Marine Strategy Framework Directive) Marine Litter Report 2011 (Descriptor 10) toolkits (Galgani et al., 2014; van Franeker et al., 2011). This involves recording the type of plastic debris: industrial plastic pellets or nurdles (*IND*) and user plastics (*USE*) which can be split into several sub-categories; sheetlike plastic (*SHE* e.g., plastic bags), threadlike/filamentous plastic (*THR* e.g., remains of rope), foamed plastics (*FOAM* e.g., polystyrene), fragments (*FRAG* e.g., hard plastic pieces) and other (*POTH*, e.g., rubber, elastics, items that are 'plastic-like', not clearly fitting into another category). Dry weight (g) was recorded for each individual piece isolated. Additional recordings of colour and three-dimensional measurements of each individual piece were also taken. Colour was recorded within 11 categories: *Clear*, *White*, *Pink/Purple*, *Red*, *Orange*, *Yellow*, *Green*, *Blue*, *Brown*, *Black*, *Grey*. Width to length were calculated (W/L) for all pieces ingested. A ratio close to 1 indicated a square or round piece of debris with ratios leading to rectangular and progressively more linear shapes with decreasing ratio. These were grouped according to five quintiles of Shape Class: *SC1* < 0.2, *SC2* < 0.4, *SC3* < 0.6, *SC4* < 0.8, *SC5* < 1.0.

2.3. Polymer identification

The polymer make-up of marine plastic debris may aid in identifying possible sources of the material. A sub-sample of randomly selected 137 (28 % of total plastic pieces) retained items was subject to analysis using Fourier-transform infrared (FT-IR) spectroscopy. This offers a simple, efficient non-destructive method for identifying and distinguishing polymers, based on infrared absorption bands representing distinct chemical functionalities present in the material (Jung et al., 2018). Analysis was carried out using a PerkinElmer Spotlight 400 universal diamond – ATR (attenuated total reflection) attachment, placing each fragment or fibre onto the diamond surface (after precleaning the surface with analytical grade ethanol) and applying a consistent force using the sample clamp. Spectra were collected over a broad range (630–4000 cm^{-1}) from an average of four sample scans with a resolution setting of 4 cm^{-1}. Spectra were corrected for background variation. The infrared spectra were acquired, processed and analysed using PerkinElmer Spectrum software (version 10.5.4.738) and compared against a total of eight commercially available polymer libraries (adhes.dlb, Atrpolym.dlb, ATRSPE~1.DLB, fibres.dlb, IntPoly.spl, poly1.dlb, polyadd1.dlb and POLYMER.DLB, as supplied by PerkinElmer), checking also against an additional library compiled at the Greenpeace Research Laboratories in order to exclude contaminants arising from materials commonly used in the laboratory. Spectrum software allowed for the comparison of spectra obtained for each sample against these nine libraries, reporting the 10 most likely matches. In each case, matches were then checked by the analyst to verify the quality of the match and the reliability of the identification. Match quality scores were generated for each spectrum, and only scores with >70 % match similarity and/or reliable spectra were accepted.

2.4. Selectivity analysis

Potential selectivity was tested for using the package "*adehabitatHS*" and graphical calculated selectivity ratios were obtained for debris type, colour and shape according to the relative makeup of debris according to these attributes in the environment (Duncan et al., 2019). A value of >1 or <1 indicates a positive or negative selectivity, respectively, compared to what is available in the environment. All data processing and analysis was performed in R version 4.4.0 (R Core Team, 2023), and the significance level for statistical tests was alpha = 0.05 throughout.

3. Results

3.1. Overall plastic ingestion

Of the post-pelagic turtles analysed (n = 131), 42.7 % (n = 57) had ingested plastic. The highest number of pieces ingested by a single individual, was 67 pieces weighing 9.66 g. Over the eleven-year period, frequency of occurrence of plastic ingestion (FO%) ranged from 0 to 70 FO% (n = 131), which did not show a significant change over time (Spearman's Correlation: $R = 0.39, p = 0.23$) or with size of turtle (CCL) ($R = 0.27, p = 0.44$; Fig. 2). When years or size classes with small sample samples ($n \leq 3$) were excluded, there were still no significant correlations in FO% (year: $R = 0.21$, $p = 0.54$; size: $R = 0.90$, $p = 0.08$).

All groups of turtles presented individuals that had ingested plastic

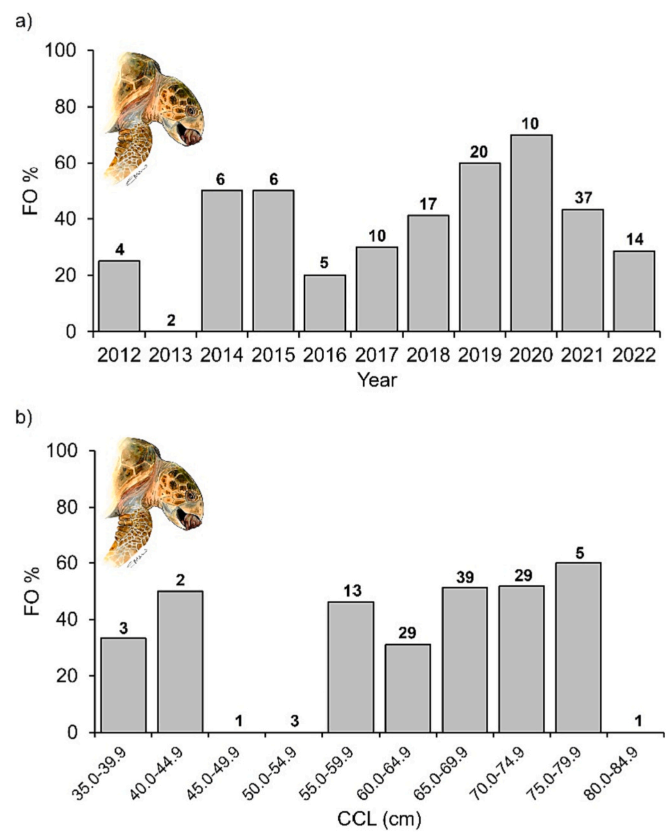

Fig. 2. Frequency of occurrence (FO%). The proportion of turtles showing plastic ingestion (n = 131) a) per year of stranding and b) according to size (n = 125). For 6 individuals CCL measurements were not known. Sample size for each increment is shown above the respective bar. Original artwork by Emma Wood.

Table 1
Abundance and mass of plastic in affected turtles. Mean ± SE and range of number and mass (g) of ingested plastic pieces of stranded and bycaught loggerhead turtles (*Caretta caretta*). For abbreviations see text.

	Number of pieces				Mass (g)			
	Stranded		Bycaught		Stranded		Bycaught	
	NLD (*n* = 31)	LLD (n = 2)	NLD (*n* = 22)	LLD (n = 2)	NLD (n = 31)	LLD (n = 2)	NLD (n = 22)	LLD (n = 2)
Mean ± SE	10.1 ± 2.8	10.0 ± 5.0	7.0 ± 1.6	1.0 ± 0.0	0.9 ± 0.3	0.7 ± 0.6	0.7 ± 0.3	0.3 ± 0.3
Range	1.0–67.0	5.0–15.0	1.0–27.0	1.0–1.0	<0.01–9.7	<0.01–1.9	<0.01–4.8	<0.01–0.6

(Table 1). There was no significant difference in incidence of plastic ingestion between NLD and LLD stranded turtles (Fisher's exact test (FET): odds ratio = 2.61, p = 0.29, n = 81), NLD and LLD bycaught turtles (FET: odds ratio = 1.42, p = 1, n = 50) and between stranded and bycaught NLD turtles (Chi-squared Test: $\chi 2$ = 0.18, p = 0.67, n = 117) and stranded and bycaught LLD turtles (FET: odds ratio = 0.45, p = 0.58, n = 14).

For plastic positive turtles, there was no significant difference in the number of pieces (Wilcoxon Rank Sum Test: $W_{33,24}$ = 419; p = 0.71) and mass of ingested plastic ($W_{33,24}$ = 361; p = 0.57) between stranded and bycaught turtles. Additionally, there was no significant difference between the number of pieces or mass of ingested plastic (g) between NLD or LLD stranded turtles (number pieces: $W_{31,2}$ = 17.5, p = 0.32; mass: $W_{31,2}$ = 21, p = 0.46), NLD or LLD bycaught turtles (number pieces: $W_{22,2}$ = 40, p = 0.07; mass: $W_{22,2}$ = 19, p = 0.79), or stranded or bycaught NLD turtles (number pieces: $W_{31,22}$ = 331, p = 0.87; mass: $W_{31,22}$ = 333, p = 0.89) or LLD turtles (number pieces: $W_{2,2}$ = 4, p = 0.22; mass: $W_{2,2}$ = 3, p = 0.67). Due to the lack of any significant differences among groups further analyses were undertaken for all plastic-positive animals combined excluding posthatchling turtles. For future reference and meta-analysis, however, data have been hosted in such a way that these different groups can be examined separately (Duncan et al., 2024a).

3.2. Patterns with body size

There was no significant relationship between turtle size (CCL) and the number of ingested pieces (Spearman's Correlation: R = −0.09, p = 0.49), mass (g) of plastic (R = 0.01, p = 0.95), and body burden index (R = −0.14, p = 0.30) (Fig. S1). There was no significant correlation between BCI and number of pieces (R = −0.05, p = 0.75), mass (g) of ingested plastic (R = −0.08, p = 0.57) or body burden index (R = 0.05, p = 0.74). Additionally, there was no significant relationship between turtle size (cm) and mean length of ingested plastic pieces (mm) (R = −0.21, p = 0.13) or maximum length of ingested plastic pieces (mm) (R = −0.11, p = 0.41) (Fig. S2).

3.3. Temporal patterns of ingestion

There were no statistically significant correlations between year and the number of pieces ingested (R = −0.13, p = 0.34), mass of ingested pieces (g) (R = 0.10, p = 0.45) or body burden index (g/kg) (R = 0.07, p = 0.63) (Fig. S1.). However, there was a significant, positive relationship between year and the maximum mass of ingested pieces (g) (R = 0.75, p = 0.01) and body burden index (g/kg) (R = 0.75, p = 0.02) (Fig. S1). Maximum number of pieces ingested demonstrated a positive non-significant trend over time (R = 0.50, p = 0.12; Fig. 3.).

3.4. Spatial pattern of ingestion

There was no significant difference between the coasts from which animals originated and abundance measures for the number of pieces (Kruskal Wallis (KW): $\chi 2_3$ = 3, p = 0.47) mass (g) (KW: $\chi 2_3$ = 2, p = 0.53) or body burden (g/kg) (KW: $\chi 2_3$ = 4, p = 0.28) of ingested plastic. Additionally, frequency of occurrence did not vary significantly by coastline (FO%: East = 42.0 %; North = 47.5 %; West = 53.8 %; Chi-squared Test: $\chi 2$ = 0.58, p = 0.75) (Table 2).

3.5. Ingested plastic description

The most abundant type of plastic ingested (n = 492) was *sheetlike* plastic (SHE: 62 %) followed by *hard fragments* (FRAG: 23 %; Fig. 4a). The most numerous colour ingested was *clear* (41 %) followed by *white* (25 %) and *black* (16 %, Fig. 4b). The majority of ingested plastic were rectangular in shape; *SC3* (31 %) followed by *SC4* (23 %, Fig. 4c).

3.6. Polymer identification

The most common polymers identified were Polypropylene (PP: 37 %; 43 %-FRAG, 36 %-THR, 32 %-SHE), Polyethylene (PE: 35 %; 47

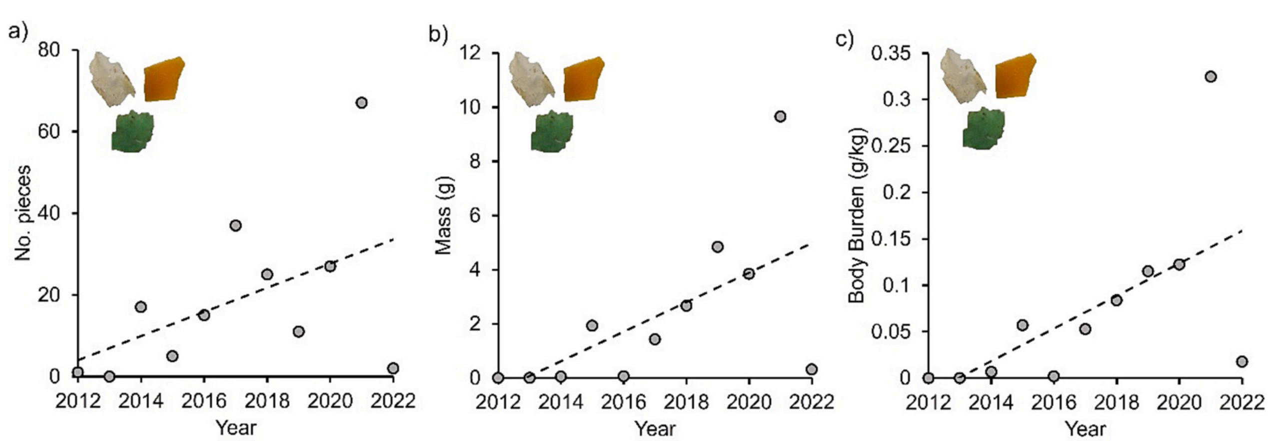

Fig. 3. Temporal pattern of maximal incidence of plastic ingestion in loggerhead turtles (n = 55). Relationship between year and a) annual maximum number of ingested plastic pieces, b) annual maximum mass of ingested plastic pieces (g) and c) annual maximum plastic debris body burden index (mg plastic/g turtle). For 2 individuals CCL measurements were not known.

Table 2
Geographic breakdown of abundance and mass of plastic in affected animals. Mean ± SE and range of number and mass (g) of ingested plastic pieces of loggerhead turtles according to the coastline from which they were retrieved.

	Location					
	East (n = 29)		North (n = 19)		West (n = 7)	
	No. of pieces	Mass (g)	No. of pieces	Mass (g)	No. of pieces	Mass (g)
Mean ± SE	8.1 ± 2.6	0.8 ± 0.4	6.2 ± 4.8	0.6 ± 0.3	11.4 ± 4.8	0.7 ± 0.3
Range	1.0–67.0	<0.01–9.66	1.0–25.0	<0.01–4.8	1.0–37.0	<0.01—1.9

%-SHE, 36 %-FRAG, 36 %-THR) and Polyamide (PA: 19 %; 27 %-THR, 21 %-FRAG, 21 %-SHE; Fig. 5.). Other polymers identified at lower levels were Polyhexamethylene (PHMB), Polyundecanoamide (PAU), Polyisoprene (PI) and Polyvinyl Chloride (PVC) (Duncan et al., 2024b).

3.7. Selectivity

There was significant selectivity of the type ($\lambda = 0.152$, $p < 0.001$), colour ($\lambda = 3.16$, $p < 0.001$) and shape ($\lambda = 0.742$, $p = 0.002$) of plastic ingested. Calculated ratios suggest loggerhead turtles exhibited a very strong selectivity towards both *sheetlike* (SHE) and *threadlike* (THR) plastic (wi = 6.97, wi = 4.52, respectively) and slight selection towards foamed (FOAM) plastic (wi = 1.93), but appeared to not actively select *hard fragments* (FRAG), *"other pollutants"* (e.g. rubber) (POTH) and industrial (IND) types (Fig. 4d). When considering the ingestion by colour categories, loggerhead turtles showed strong selectivity for *clear* and *black* debris (wi = 1.99, wi = 1.95, respectively) and also highly marginal selectivity for *pink/purple* (wi = 1.01), while not showing active selection of *white*, *red*, *grey*, *orange*, *blue*, *brown*, *yellow*, *green* plastics (Fig. 4e). For shape, the strongest selectivity was towards thin, elongated pieces *(SC1)* and a very slight selectivity towards more rectangular shapes *(SC3)* (wi = 2.74, wi = 1.13, respectively; Fig. 4f).

3.8. Plastic ingestion in posthatchlings

Plastic ingestion in posthatchling turtles ($n = 4$) was at a frequency of occurrence (FO%) was 75 % with the mean number pieces being 7.0 ± 5.5 (mean ± SE; range: 1-18) and mass (g) ingested was 0.11 ± 0.10 (range: 0.001-0.31). The most prevalent type of plastic ingested for posthatchling turtles was *sheetlike* plastic (SHE: 77 %) followed by *foamed plastics* (FOAM: 14 %) and *hard fragments* (FRAG: 9 %). The main colours ingested were *white* (32 %), *clear* (32 %) and *black* (14 %). Most of the ingested plastic were in *SC4* (32 %) followed by *SC3* (23 %). For ingested pieces from one individual posthatchling of 18.8 CCL (cm) that received polymer analysis all pieces were identified as Polyamide ($n = 7$).

4. Discussion

Here we make a significant addition to the limited knowledge base regarding plastic ingestion in loggerhead turtles within the data poor region of the eastern Mediterranean basin with the high contribution of fresh bycaught turtles. This allowed detailed comparisons between stranded turtles and those bycaught directly from foraging grounds. Loggerhead turtles in the study exhibited plastic ingestion with frequency of occurrence (FO%) in the mid-range of values for those reported previously in the Mediterranean (14-85 FO%; (Camedda et al., 2014; Casale et al., 2008; Digka et al., 2020; Lazar and Gračan, 2011; Matiddi et al., 2017; Tomas et al., 2002). The majority of ingestion was of sheet-like plastics, with potential sources being plastic bags and food packaging, which is consistent with results from previous reports from other regions and species of marine turtle (Lynch, 2018; Schuyler et al., 2014b). Furthermore, colours were also similar to those previously reported; clear, white and black being the majority of those found (Lynch, 2018; Schuyler et al., 2014b). The dominance of Polyethylene and Polypropylene as polymers found also follows global trends (Bruno et al., 2022; Camedda et al., 2022a; Solomando et al., 2022). For post-pelagic turtles there was no relationship between turtle size and plastic ingestion abundance measures which is in accord with previous work in the western Mediterranean (Casale et al., 2016). This may, in part, be due to size being a poor predictor of loggerhead turtle foraging ecology in the Mediterranean due to the proximity of different habitats and smaller turtles foraging benthically at comparably smaller sizes to others globally (Casale et al., 2008).

Plastic ingestion FO% and abundance measures did not show a marked increase over the 11-year time period of this study. This is consistent with other recent long-term studies within the region (Darmon et al., 2022). Plastic ingestion could depend on food and litter availability, which varies spatially, seasonally and annually (Darmon et al., 2022; Mansui et al., 2020) obfuscating temporal trends, with relatively small sample sizes per year. Despite this, it is important to note the maximum values of abundance estimates here did increase over the study period, perhaps indicating the extreme cases of ingestion are increasing temporally. Other regional studies on different species with longer time series data have noticed a marked increase overtime from minimal or no plastic ingestion in the 1970-90s to higher rates of occurrence (Mrosovsky et al., 2009; Yaghmour et al., 2021). As environmental plastic levels were already very high in the early to mid-1990s in the eastern Mediterranean (Broderick and Godley, 1996), detecting a similar increase in plastic ingestion in our dataset as observed in the wider literature is likely difficult based on the time series of our study (Broderick and Godley, 1996).

Previously the literature has called for only turtles assumed to have had normal feeding behaviour to be taken in consideration as plastic ingestion occurrence in stranded turtles may be biased as consequence of illness or injury, with health status modifying the normal foraging behaviour (Casale et al., 2016). A recent large-scale study, which included twenty four animals also included in the current work, however, found that bycaught and stranded exhibited no difference in body condition (Darmon et al., 2022). Within the current study we observed no difference in ingestion incidence or abundance measures between those turtles defined as stranded or bycaught. Indeed, there was also no difference in recent dietary composition and feeding behaviour between these two groups of loggerhead turtles in this study area (Palmer et al., 2021). It is important to note within this study area that many strandings are thought the result of fisheries interactions (Snape et al., 2013), since strandings are temporally correlated with setting of high bycatch fishing metiers during months when fishers indicated bycatch is a problem and because in the majority of cases, the only cause of death is circumstantial evidence indicating drowning in fisheries. The mortalities within this area are thought to happen in shallow, near-shore waters due to interaction with small-scale/semi-industrial fishing fleets, with the greatest proportion of fisheries deaths occurring in set nets (Palmer et al., 2024; Snape et al., 2013, 2016). Another consideration we investigated was the inclusion of a limited number of turtles with low levels of dietary digesta. Again, no marked differences in plastic ingestion incidence or abundance were apparent according to this factor. Sample sizes for some groups were, however, small, and ongoing consideration should be given to these potential sources of bias.

Although differing classification systems of plastic prohibit detailed

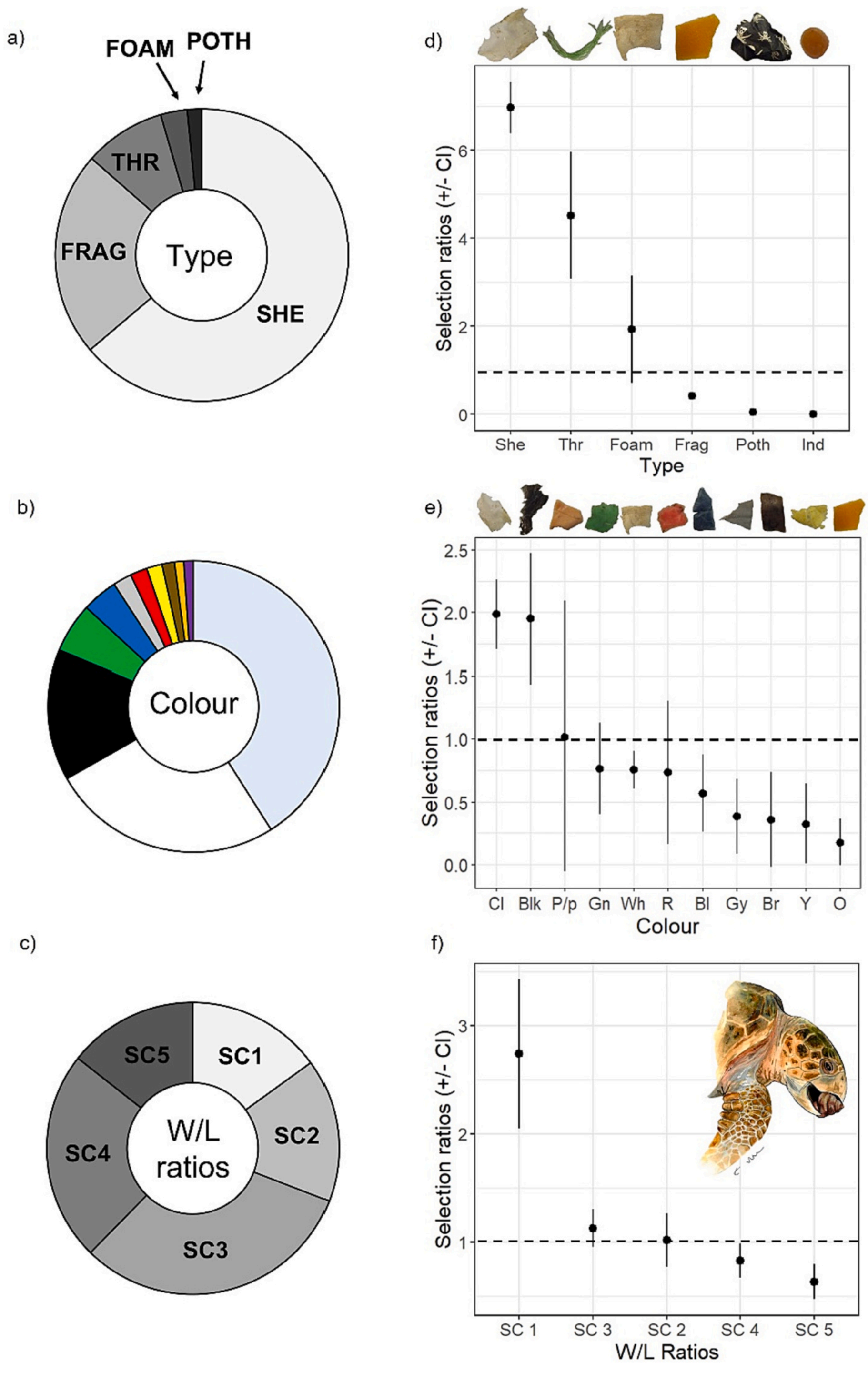

Fig. 4. Marine turtle diet-related selectivity in macroplastic ingestion in the loggerhead turtles (*Caretta caretta*) ($n = 57$) a) & d) type of plastic debris SHE = sheetlike plastics, THR = threadlike plastics, FOAM = foamed plastics, FRAG = hard plastics, POTH = other 'plastic like' items, IND = industrial nurdles, b) & e) colour of plastic debris. Cl = Clear, Blk = Black, Y = Yellow, Wh = White, Gn = Green, Bl = Blue, Br = Brown, Gy = Grey, O = Orange, P/P = Pink/Purple, R = Red, c) & f) width/length ratio (WL ratio). If the ratio number produced was <0.2 this represented linear/rectangular shape whereas a ratio close to 1 indicated a more square or circular piece of debris. Shape classes are as followed; SC1 = 0.01–0.2, SC2 = 0.21–0.4, SC3 = 0.41–0.6, SC4 = 0.61–0.8, SC5 = 0.81–1.0. d), e) & f) Selectivity Ratios. A value >1 this indicates a positive selectivity for that type/colour category than availability in the environment. Error bars indicate 95 % confidence intervals and ranked from strongest to weakest selection ratios. c) d) & f) Classification of ingested plastic from proportion (%) of plastic pieces. Original artwork by Emma Wood. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

comparisons to some previous studies of debris selectivity in other populations (Schuyler et al., 2012), green turtles (*Chelonia mydas*) from the eastern Mediterranean exhibited similar selectivity as loggerhead turtles in this study, with strong selections towards sheet-like and thread-like plastic, being clear or black in colour with small length to width ratios (Duncan et al., 2019). Although the selectivity for green items demonstrated for green turtles by Duncan et al. (2019) was not shown here. These patterns are contrary to what might have been expected as a result of species-specific foraging behaviour and dietary preferences (Palmer et al., 2021). Despite loggerhead turtles ingesting a

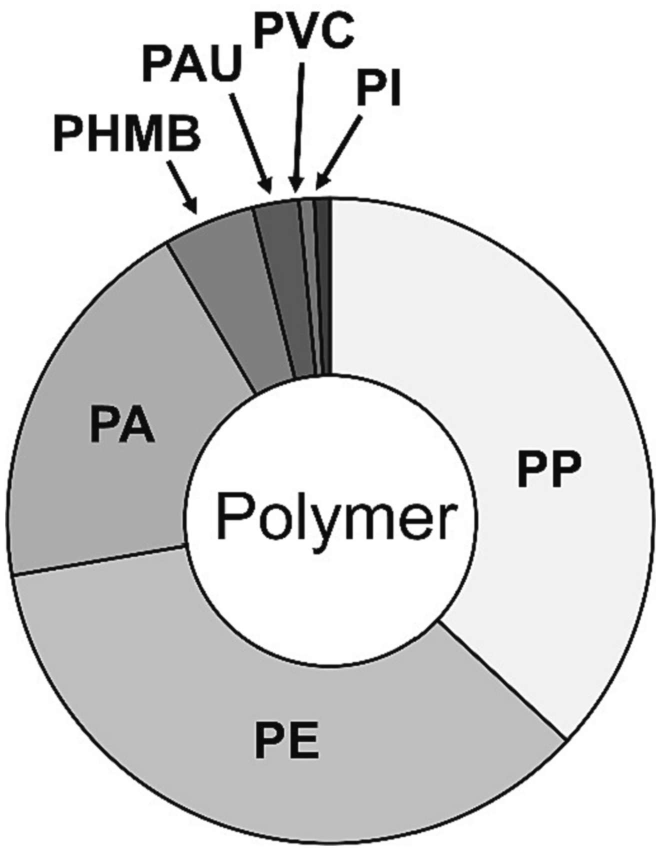

Fig. 5. Polymer identification of ingested plastic ($n = 130$) from 33 loggerhead turtles (*Caretta caretta*) in Cyprus. Proportion (%) of ingested pieces: PE, Polyethylene; PP, Polyproplene; PA, Polyamide; PHMB, Polyhexamethylene; PAU, Polyundecanoamide; PI, Polyisoprene; and PVC, Polyvinyl Chloride.

higher proportion of harder fragments and items such as bottle top lids, that could be confused with hard bodied prey items, than green turtles in this region (Duncan et al., 2019), they did not appear to actively select these, possibly an artifact of the extremely high abundance of fragments in the environment (Duncan et al., 2018).

Post-hatchling sized turtles showed high incidence of plastic ingestion, however, the small sample size of this size class prohibited any further analysis but does add evidence for the concern for vulnerability for this life stage (Pham et al., 2017a; Rice et al., 2021; White et al., 2018). Previous studies have reported high occurrences and plastic present in this marine turtle life stage and suggested this as a potential evolutionary trap, with signs of morbidity and mortality presenting as evidence of impacts such as gastrointestinal ulceration on necropsy (Duncan et al., 2021). Life stage is likely be an important factor in selectivity with post-hatchling turtles varying in not only their likelihood of plastic ingestion but also being less selective and more opportunistic in their dietary choices (Schuyler et al., 2012; Schuyler et al., 2014a, 2014b). However sample size and ability to collect environmental baselines for this life stage often precludes them from detailed analysis (Duncan et al., 2021; Pham et al., 2017b; Rice et al., 2021; White et al., 2018). Further insights into the impacts of this rarely encountered life stage will necessarily come from multiple research groups combining sparse data.

Although bycaught and stranded turtles did not differ in patterns of plastic ingestion within this study, origin of turtles sampled should be maintained as a careful consideration within marine turtle plastic ingestion studies. There is strong indication that different foraging behaviors could be represented in sample groups caught in different types of gear (e.g. pelagic longline and trawl nets; Casale et al., 2016), impacting the patterns of plastic ingestion. This is due to the close link between gear types, habitat, diet and foraging behaviour (Palmer et al., 2021). For example, turtles captured in pelagic longlines are likely to be of a life stage tending to feed on epipelagic prey in comparison to other life stages that target prey benthically being more prone to interaction with set nets. Here the environmental baseline data was derived from beach plastic surveys as they were the only logistically feasible way to collect a baseline proxy. Limitations are recognised to using this method as the resulting composition of plastic present on beaches can vary to other environment compartments, but do provide a cost-effective, widely accessible and a comprehensive method to estimate when at-sea sampling is not possible (Di Beneditto and Awabdi, 2014; Duncan et al., 2019; Schuyler et al., 2012). Sampling in known marine turtle foraging areas remain a priority activity to help inform such studies.

Currently marine turtles are proposed as an indicator species for monitoring marine litter in the Mediterranean region (Camedda et al., 2022a; Darmon et al., 2022; Solomando et al., 2022). Monitoring this region is important as the Mediterranean Sea shows non-homogeneous values for plastic pollution in space and time, likely resultant from geographic variability in levels of plastic input and the existence of non-permanent eddies (Constantino et al., 2019; Mansui et al., 2015). The EU Marine Strategy Framework Directive (MSFD) has including "Trends in the amount and composition of litter ingested by marine animals" among its indicators (Commission's Decision 2010/477/EU) (Galgani et al., 2014). Green turtles in the eastern Mediterranean have shown higher FO% and ingestion abundance measures than loggerhead turtles stranded or bycaught within the same area (Duncan et al., 2019). Similar interspecific differences (FO%, number of pieces and mass of ingested plastic) have been noted in the United Arab Emirates, with green turtles ingesting higher quantities of marine debris than loggerhead turtles (Yaghmour et al., 2021). Using only the loggerhead turtle as an indicator species in the eastern Mediterranean may give a skewed view of the current state of plastic pollution in this region (Darmon et al., 2022) as eastern Mediterranean environments experience plastic values among the highest levels reported globally (Duncan et al., 2018).

Loggerhead turtles have been suggested to be used to verify the effectiveness of the Single-use Plastic Directive (EU 2019/904) and patterns of ingestion presented here underscore this utility. Plastic bags and food packaging are among some of the most observed litter items in the Mediterranean Sea (Arcangeli et al., 2018; Constantino et al., 2019) and these map to the materials ingested by loggerhead turtles within this study and others (Camedda et al., 2022b; Darmon et al., 2022). Further work is needed here to understand the processes leading to ingestion. For example, questions arise as to whether marine turtles bite small thin pieces of larger items when they interact with them, possibly explaining the dominance of similar sheet-like pieces in the same samples. Therefore, the utilisation of technology such as mounted camera tags on turtles will improve knowledge of turtle/plastic interactions (Fukuoka et al., 2016). Additionally, olfactory stimuli might also play an important role on the ingestion of marine debris by turtles (Pfaller et al., 2020) and is worthy of further investigation.

Overall loggerhead turtles in the eastern Mediterranean showed consistent and considerable plastic ingestion over the course of an 11-year period. Due to the widespread distribution of this species, with harmonised monitoring implemented at a wide spatial scale they could be considered a good bio-indicator species (Galgani et al., 2014; Matiddi et al., 2017), albeit with larger sample sizes needed. It will, however, be important to carefully consider interspecific differences and to make sure a true representation of environmental status is reflected and, we suggest, green turtles are also considered. Further efforts should be made towards increased standardisation of methodologies to continue monitoring this threat alongside investigations into the potential lethal and sublethal impacts of plastic on the health and survival of marine turtles within this region and worldwide (Marn et al., 2020).

CRediT authorship contribution statement

Emily M. Duncan: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Hasan Deniz Akbora:** Writing – review & editing, Data curation. **Patrizia Baldi:** Writing – review & editing, Data curation. **Damla Beton:** Writing – review & editing, Project administration, Funding acquisition, Data curation. **Annette C. Broderick:** Writing – review & editing, Funding acquisition, Data curation, Conceptualization. **Burak Ali Cicek:** Writing – review & editing, Data curation. **Charlotte Crowe-Harland:** Writing – review & editing, Data curation. **Sophie Davey:** Writing – review & editing, Project administration, Data curation. **Tess DeSerisy:** Writing – review & editing, Data curation. **Wayne J. Fuller:** Writing – review & editing, Data curation. **Julia C. Haywood:** Writing – review & editing, Data curation. **Yu Jou Hsieh:** Writing – review & editing, Data curation. **Ecem Kaya:** Writing – review & editing, Data curation. **Lucy C.M. Omeyer:** Writing – review & editing, Data curation. **Meryem Ozkan:** Writing – review & editing, Data curation. **Josie L. Palmer:** Writing – review & editing, Data curation. **Emma Roast:** Writing – review & editing, Data curation. **David Santillo:** Writing – review & editing, Methodology, Data curation. **M. Jesse Schneider:** Writing – review & editing, Data curation. **Robin T.E. Snape:** Writing – review & editing, Project administration, Funding acquisition, Data curation, Conceptualization. **Katrina C. Sutherland:** Writing – review & editing, Data curation. **Brendan J. Godley:** Writing – review & editing, Writing – original draft, Resources, Project administration, Methodology, Funding acquisition, Data curation, Conceptualization.

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

doi still pending from the data repository (PANGAEA)

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Appendix A. Supplementary data

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