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Litter ingestion and entanglement in green turtles: An analysis of two decades of stranding events in the NE Atlantic☆

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ABSTRACT

Survivorship of early life stages is key for the well-being of sea turtle populations, yet studies on animals that distribute around oceanic areas are very challenging. So far, the information on green turtles (*Chelonia mydas*) that use the open NE Atlantic as feeding grounds is scarce. Strandings occurring in oceanic archipelagos can provide relevant information about the biology, ecology and current anthropogenic pressures for megafauna inhabiting the open ocean. In this study, we analysed stranding events of green turtles found in the Azores archipelago to investigate interactions with marine litter. In addition, we quantified and characterized litter items stranded on beaches to provide a direct comparison between the ingested items with the debris found in the environment. A total of 21 juvenile green turtles were found stranded in the region between 2000 and 2020 (size range: 12–49 cm, CCL). Overall, 14% of the animals were entangled in marine litter and 86% of the turtles necropsied had ingested plastic. The mean abundance of items ingested was 27.86 ± 23.40 and 98% were white/transparent. Hard plastic fragments between 1 and 25 mm were the most common shape recovered in the turtles, similarly to what was found on the coastline. All of the litter items analysed with pyrolysis GC-MS revealed to be polyethylene (PE). This study provides the first baseline assessment of interactions of plastic litter with juvenile green turtles found at the east edge of the North Atlantic Subtropical Gyre. The combination of these results supports the hypothesis that migratory megafauna that use remote oceanic islands as a feeding ground are exposed to anthropogenic litter contamination dominated by plastics, even when these regions are located far away from big industrial centers or populated cities.

1. Introduction

Sea turtles are characterized by having a broad distribution, complex life cycles, slow growth rates, and delayed sexual maturity (e.g. Musick and Limpus, 1997; Bolten, 2003a), but basic aspects of their life history such as location, duration and ecology of early stages remain poorly understood (Bolten, 2003a; Mansfield and Putman, 2013). They play an important ecological role in oceanic and coastal marine ecosystems (e.g. Frazier, 2005; Kinan and Dalzell, 2005), yet most of the species are recognized as threatened in the International Union for Conservation of Nature's (IUCN) Red List of Threatened Species (www.iucn.org; last accessed May 2021).

Chelonia mydas, commonly known as the green turtle, is the largest of the six species of hard-shelled sea turtles. They are usually found near coastal areas in temperate, subtropical and tropical waters throughout the world's oceans (Seminoff et al., 2015). Green turtles are rarely encountered during the first years of life, and thus are challenging to study after their rapid movements from nesting beaches to areas in the open ocean (Carr and Meylan, 1980; Carr, 1987; Reich et al., 2007). In the early oceanic-stage green turtles are omnivorous. When they undergo their ontogenetic transition from oceanic to neritic habitats, green turtles become primarily herbivorous (Bjorndal, 1997; Arthur et al.,

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2008; Esteban et al., 2020).

Throughout their geographic range, green turtles have been affected by threats such as bycatch, climate change, human exploitation (meat and eggs as food resource), habitat degradation and various types of pollution including marine litter (Hawkes et al., 2009; Bolten et al., 2011; Lewison et al., 2014; Schuyler et al., 2014). Currently, marine litter (composed mainly of plastic) is having dire consequences for marine ecosystems and wildlife (e.g. Barnes et al., 2009; Bergmann et al., 2015; Canals et al., 2020). Since the first published accounts of plastic contamination in the marine environment in the early 1960s, an increasing interest and effort in this field have revealed interactions in a large diversity of marine organisms. So far, more than 900 species are known to have been affected by a wide range of problems through entanglement in, or ingestion of, litter items (Kühn and van Franeker, 2020). However, no study has yet quantitatively demonstrated population-level impacts on marine megafauna (Senko et al., 2020). Nelms et al. (2016) recommended that future studies of interactions between marine litter and sea turtles should be focused in animals distributed along convergence areas, where high densities of plastics are more likely to occur. Particularly, the Central North Atlantic was considered among the pelagic areas where the effort to assess plastic ingestion in sea turtles should receive particular attention (Lynch, 2018).

Due to the numerous threats that affect green turtles worldwide, research and conservation efforts have been increasing (Rees et al., 2016), but certain foraging areas remain poorly understood. For example, while the Canary Islands have been considered the northernmost habitat for populations of resident green turtles in the NE Atlantic (Carreras et al., 2014; Monzón-Argüello et al., 2018), juvenile green turtles are commonly observed in the archipelago of the Azores further north. However, research on this aggregation has been scarce due to their cryptic behavior that has hampered the collection of information to evaluate their role in the surrounding ecosystem.

The aim of this study was to investigate the interaction of marine litter with juvenile green turtles in the NE Atlantic. We analysed stranding data of green turtles in the Azores archipelago over 21 years (2000–2020), in combination with information collected from necropsies of dead turtles. We also performed ten beach surveys along the entire region to estimate the composition of the litter present in the marine environment, in an attempt to understand the mechanisms of plastic ingestion.

2. Materials and methods

2.1. Study area

The Azores is an archipelago composed of nine volcanic islands (Fig. 1), characterized by an oceanic, subtropical climate and ocean surface temperatures varying between 15 °C and 27 °C (Amorim et al., 2017). Located at the eastern edge of the North Atlantic Subtropical Gyre (NASG), the Azores has a great abundance of resident animals, but it is also a transitional habitat for large open-ocean species such as marine mammals, sharks, sea turtles and seabirds (Afonso et al., 2020). In the Azores, the North Atlantic Current (NAC) strongly influences northern areas, whereas southern regions are under the effect of the Azores current (AC). The region is characterized by an extensive Exclusive Economic Zone (EEZ: ~1 million km^2), with a seafloor composed mainly of abyssal areas that cover more than 85% of the EEZ and that reach depths of 5000 m (Perán et al., 2016). The islands are located on both sides of the Mid-Atlantic Ridge and are scattered on the intersection of three tectonic plates (the North American Plate, the Eurasian Plate and the African Plate), with around 600 km of ocean between the two most distant islands (Corvo and Santa Maria). The sublittoral area of the Azores represents only 0.05% of the seafloor territory (Perán et al., 2016), indicating that suitable benthic habitats for green turtles are spatially restricted in this region.

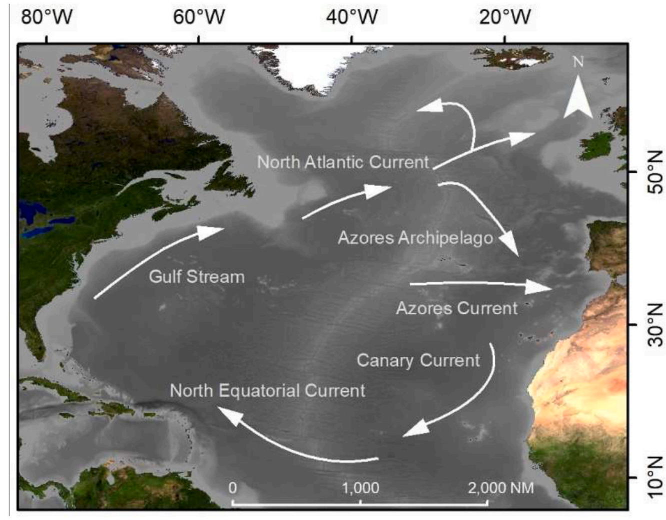

Fig. 1. The Azores archipelago located at the edge of the North Atlantic Subtropical Gyre (NASG).

2.2. Data collection

We analysed data collected by the Regional Stranding Network in the Azores, locally known as “Rede de Arrojamento de Cetáceos dos Açores” (RACA). RACA was established in 1999 to; a) minimize the possible threat of strandings to human health and safety, b) minimize the pain and suffering of live stranded animals and, c) increase scientific and educational benefits of stranded animals. RACA is coordinated by the Regional Coordination Center of the Direção Regional dos Assuntos do Mar (DRAM). It further relies on a network of institutional partners, including Maritime Authorities (National Guard - GNR/SEPNA and Maritime Guard - Policia Maritima), which organize in local coordination centers on each island and the Island Natural Parks - Operational Centers. In addition, this network also cooperates with a wide variety of stakeholders including veterinarians, tourist operators, professional fishers, volunteers and scientists.

Standardized morphometric measurements were taken using a flexible tape measure (curved measurements) and a calliper (straight-line measurements) following Bolten (1999). Overall, the prime size measurement used was the minimum curved carapace length (CCL; Table 1), which is measured from the anterior point at midline, or nuchal scute, to the posterior notch at midline between the supra caudals. Injured sea turtles were generally transported to an aquarium facility on Faial Island for rehabilitation and later returned to the wild. Dead animals were either preserved frozen for later necropsy or buried. Depending upon the circumstances of the stranding events and the decomposition status of dead sea turtles, up to six different external measurements were recorded (Table S1). Thus, measurements taken during necropsies included CCL and SCL (measured from the nuchal scute to the posterior notch at midline between the supracaudals) and CCL n-t and SCL n-t (measured from the nuchal scute to the posterior tip of the supracaudals). Curved carapace width (CCW) and straight carapace width (SCW) were measured at the widest point. Location was identified as the island where the animal stranded. Finally, entanglement in marine litter and marine litter ingestion (detected after necropsies of dead animals or by defecation of rehabilitated animals) were noted.

2.3. Necropsy methodology and marine litter classification

Dead sea turtles were necropsied following a standardized protocol (Matiddi et al., 2019). During the necropsies, the gastrointestinal tract (hereafter GI) was isolated and divided into three different sections (esophagus, stomach, intestines). The content of each section was

Table 1
Green turtles found stranded in the Azores since 2000. Size (CCL) is provided as CCL min (curved carapace length measured from the nuchal scute to the posterior notch at midline between the supracaudals). The stranding locations are specified as follow; Faial (FAI), Terceira (TER), Graciosa (GRA), São Miguel (SMG), Flores (FLO), Santa Maria (SMA), Corvo (COR) and Pico (PIC). Status of the stranded animals is assigned by RACA to one of three groups: animals that are found dead (DEAD), animals that are found alive but did not survive (A/D), and animals that are found alive, rehabilitated and released (ALIVE). Entangled in abandoned, lost or discarded fishing gear (ALDFG) is also identified.

Year	Month	Status	Actions	CCL (cm)	Island	Human interactions
2001	May	DEAD	Necropsy	30.7	FAI	Plastic ingestion
2008	Nov.	DEAD	Necropsy	32	FAI	Plastic ingestion
2008	July	ALIVE	Release	n.a	TER	
2009	Jan.	A/D	Advance decomp./ Buried	n.a	GRA	
2009	May	ALIVE	Release	n.a	SMG	
2009	May	DEAD	Necropsy	26.2	FAI	Plastic ingestion
2011	July	DEAD	Advance decomp./ Buried	n.a	SMA	
2013	April	DEAD	Advance decomp./ Buried	n.a	FLO	
2013	June	ALIVE	Release	n.a	SMA	Entanglement in ADLFG
2014	May	DEAD	Advance decomp./ Buried	37	SMG	
2015	Oct.	DEAD	Advance decomp./ Buried	47	SMG	Carapace injuries (Likely boat collision)
2016	April	A/D	Rec. Facility/ Necropsy	28	FAI	Plastic ingestion
2017	March	A/D	Rec. Facility/ Necropsy	12.1	FAI	Plastic ingestion
2017	–	DEAD	Necropsy	39.5	FAI	Plastic ingestion
2017	Aug.	A/D	Necropsy	49	SMG	
2017	Aug.	ALIVE	Rec. Facility/ Release	25	FAI	Plastic defecation
2017	Aug.	ALIVE	Rec. Facility/ Release	24	COR	Entanglement in ADLFG
2017	Aug.	ALIVE	Release	20	PIC	
2019	Sept.	DEAD	Advance decomp./ Buried	50	SMG	
2019	Sept.	ALIVE	Release	32	SMA	Entanglement in ADLFG
2020	July	A/D	Unknown	29.5	TER	

filtered separately using a sieve with a mesh of 1 mm. Natural diet was identified and recorded. Pieces of litter were counted, weighed (dry mass, ±0.001 g) and measured (longest dimension, ± 0.01 mm). Plastic items were grouped into three different size groups: large microplastic (1–5 mm), mesoplastic (>5–25 mm) and macroplastic (>25 mm). Litter items were also classified into seven different shapes: industrial plastic pellets also known as nurdles (IND PLA), hard plastic fragments (USE FRA), sheet-like items or soft plastics (USE SHE), threadlike items composed mainly of items related to fishing activities such as ropes and fishing lines (USE THR), foamed plastics (USE FOA), other plastics (USE POTH) and litter other than plastic (OTHER) (Matiddi et al., 2019). Finally, 11 different colours (white/transparent, black, blue, brown, green, grey, orange, pink, purple, red and yellow) were recorded for litter fragments and three colours were recorded for plastic pellets (white/transparent, black and coloured) following the classification adopted by Pham et al. (2020).

The typology of polymers ingested by green turtles were analysed for a sub-sample of plastics (5%) using Pyrolysis-gas chromatography/mass spectrometry (Pyrolysis GC-MS). This methodology is one of the most accurate methods used in the determination of chemical compositions of polymeric materials. A two-tier approach comprised of unique fingerprints of marker peaks and mass spectra embedded within each peak provided additional confidence in the data quality. For each item, a small piece (less than 1 mg mass) was placed in a quartz tube (2.5 mm OD/1.9 mm ID x 25 mm L), which had been heat-cleaned three times at 1200 °C. The quartz tube was placed in the platinum coil of CDS-2000 Pyroprobe, which was inserted into CDS-1500 Valved GC Interface maintained at 320 °C. The sample was pyrolyzed by heating the platinum filament at 650 °C for 5 s. One sample was pyrolyzed at 750 °C, which provided identical results. The pyrolysis products were analysed by using Agilent 6890N GC/DB-5 (0.25 mm OD x 60 m L; 0.25 μ film thickness) fused-silica capillary column and Agilent 5973 MSD. Column temperature was held at 45 °C for 2 min, ramped to 320 °C at 20 °C/min, and held at 320 °C for 19 min with a run time of 34.75 min. Hydrogen carrier gas flow rate was 1.2 ml/min in a constant flow mode. The CDS-1500 Interface, GC Inlet, MSD Transfer Line were kept at 320 °C. Injector split ratio was 20:1. Data acquisition used a full-scan mode from 29 to 600 amu.

2.4. Beach litter sampling

Beach litter surveys were conducted at ten different sandy beaches in April 2019 using a sampling methodology specifically designed for small litter items (1–25 mm). This approach has been used as a proxy to assess marine litter availability in the environment (e.g. Schuyler et al., 2012; Duncan et al., 2019; Acampora et al., 2014). The beaches were located on six islands (Faial, Corvo, Flores, Terceira, São Miguel and Santa Maria). We sieved the first centimeter of sand within 50 cm*50 cm quadrats using metal sieves with a mesh size of 0.9 mm. Eight replicates were collected along two levels of the shore (4 replicates per level), namely the spring high tide and the last high tide (see details on the methods in Pham et al., 2020). In the laboratory, litter items present in each sample were sorted and classified by size, colour and shape, following the same scheme as for the turtles' gut contents.

2.5. Data analysis

Percentage frequency of ingestion (%FO) was defined as the number of turtles with plastic items recovered from the GI divided by the total number of turtles analysed (n = 7). The Jeffrey's method was applied to calculate Confidence Intervals (CI) using 95% of confidence (Provencher et al., 2017). The population average (Kühn and van Franeker, 2020), also known as average plastic abundance, was calculated by dividing the number of items and the mass of items ingested by the total number of animals analysed in this study (thus, including animals in which plastics were not present), using the standard deviation (±SD) and the standard error of the mean (±SE) as recommended by Provencher et al. (2017). Plastic load (also known as intensity) was calculated using the number of items and the mass of items ingested divided by the animals that had plastics in the GI (Provencher et al., 2017). Median values were also reported. Body burden was calculated as g of plastic/kg of turtle to standardize the ingestion of marine litter per turtle mass (Lynch, 2018). Data analyses were performed using the R software (R Core Team, 2020).

In order to investigate if the composition (shape and colours) of plastic was similar between what was found inside the turtles and stranded on beaches, a permutational multivariate analysis of variance (PERMANOVA with 999 permutations; Anderson et al., 2008) was performed with square-root transformed data. These data were plotted using a Non-Metric Multidimensional Scaling (nMDS) approach based in a ranked distance or dissimilarity (Bray-Curtis coefficient). These analyses were done in PRIMER v6 software.

3. Results

3.1. General information and biometrics

The stranding network in the Azores collected data between 2000 and 2020 from a total of 185 sea turtles, of which 21 were green turtles (Table 1). Stranding events of green turtles were recorded on 8 of the 9 islands of the Azores (Fig. S1). Most of them were recorded on the island of Faial (32%), followed by São Miguel (23%), which is the largest and most populated island in the archipelago. Size could only be recorded for 15 individuals (Table 1). Average CCL was 32.1 cm (±10.8, SD; ± 2.8, SE), ranging from 12.1 cm to 49.0 cm (Table 1). Nine of the stranded turtles were found dead; the remaining animals found alive were either released after a recovery period (n = 7), or eventually died (n = 5). Most stranded animals were found during the summer months (n = 9), spring (n = 7) and autumn (n = 4) while only a single green turtle was found stranded during the winter.

Out of the turtles that were dead upon collection (n = 14), seven were necropsied. Six of the necropsied turtles were found stranded on Faial, whereas the last animal was found on São Miguel. The six turtles found on Faial were less than 40 cm CCL while the one from São Miguel was 49 cm (Table S1). The necropsy confirmed that the individual from São Miguel presented clear symptoms of disease (organs with blackish colour and very strong smell despite that the animal was fresh). The mean biometric values for the necropsied turtles were 31.1 cm for CCL (±11.4 SD; ± 4.3 SE) and 29.1 cm for SCL (±10.9 SD; ± 4.1 SE). Mean CCW was 25.9 cm (±10.2 SD; ± 4.2 SE), while the mean SCW was 20.2 cm (±10.4 SD; ± 4.6 SE). Necropsied turtles had a mean mass of 4.56 kg (±4.90 SD; ± 2.19 SE).

Natural diet items were found along the GI in 71% of the animals (40% of the stomachs and 80% of the intestines). High quantities of algae, mainly represented by *Sargassum* spp., were found in 57% of the turtles. Animal matter was also ingested and was represented by four different groups: a pteropod, a fish skeleton and a shell of a sea urchin. In addition, remains of a gelatinous organism were found in the stomach of the smallest turtle (12.1 cm CCL min).

Overall, evidence of human interactions was detected in 52% of the stranded green turtles; 90% of these events were related with marine litter, while one individual showed signs of boat collision.

3.2. Interactions with marine litter

3.2.1. Entanglement in marine litter

Entanglement in marine litter was detected in three stranded green turtles (14% FO), all of which were entangled in plastic items related with fishing activities, specifically in abandoned, lost or discarded fishing gear (ALDFG). All the entangled animals were released, one of them after a period in the recovery facility to treat damages to the right flipper. These entanglements were detected in different years (2013, 2017 and 2019), and on two islands with considerable distance between them (Corvo and Santa Maria).

3.2.2. Ingestion of marine litter

All marine litter items recovered from the GI were plastic (Fig. 2). The %FO of plastic ingestion in the necropsied turtles (n = 7) was 86%, with the lower and upper Jeffrey's confidence interval (CI) being 50% and 98%. The average abundance of plastic items per turtle was 27.86 (±61.91 SD; ± 23.40 SE), ranging from 0 to 168 pieces per turtle (Table S2). The median number was 7 items per turtle (Q1 = 1.00; Q3 = 10.00). Plastic load was 32.50 items (±66.47 SD; ± 27.14 SE) per turtle, with a median number of 7 items per turtle (Q1 = 1.75; Q 3 = 49.50). The average number of items found in the stomachs were 0.83 items (±1.60 SD; ± 0.65 SE), whereas an average of 31.67 items (±66.87 SD; ± 27.30 SE) were recovered from the intestines. The percentage of plastic items found per organ was 3% in the stomachs and 97% in the intestines. We did not find any plastic item in the esophagus section. The average mass of plastic was 0.301 g (±0.55 SD; ± 0.21 SE) per turtle, with a range between 0.000 and 1.526 g. The median mass was 0.051 g of items per turtle (Q1 = 0.002; Q3 = 0.309). The load mass was 0.351 g (±0.59 SD; ± 0.24 SE) per turtle, with a median mass of 0.128 g per turtle (Q1 = 0.012; Q3 = 0.613). Average body burden was 0.519 g/kg (±0.55 SD; ± 0.28 SE; range 0.0004–1.1714 g/kg), calculated only for the necropsied turtles that ingested plastic and had body mass available (n = 4).

Hard plastic fragments were the most frequent shape of plastic ingested (USE FRA; 96%, n = 187). The remaining items were divided between plastic pellets (IND PLA; 1.0%, n = 2), soft plastics (USE SHE; 1.5%, n = 3), threadlike items (USE THR; 1.0%, n = 2) and foamed plastics (USE FOA; 0.5%, n = 1) (Fig. 3a). Regarding main colours, 98.5% (n = 192) of the total number of plastic items recovered were white or transparent, and 1.5% (n = 3) was divided between blue, black and red colours (Fig. 3b).

The mean length of all plastics ingested was 5.43 mm (±3.55 SD; ± 0.25 SE). The median length was 5 mm (Q1 = 3; Q3 = 7). The smallest item detected was 1 mm in length, whereas the largest item had a length of 30 mm. Both were white plastic fragments recovered from the intestines. Overall, large microplastics represented 62% (n = 120) of the sample, 37% were mesoplastics (n = 73) and 1% were macroplastic items (n = 2) (Fig. 4).

Pyrolysis GC-MS pyrograms for a sub-sample of plastics isolated from the green turtles' GI displayed similar and unique fingerprints characteristic of polyethylene polymer (PE) (Fig. S2). The pyrograms contained a series of evenly spaced clusters of hydrocarbons, with each successive cluster containing one additional carbon atom than the previous cluster.

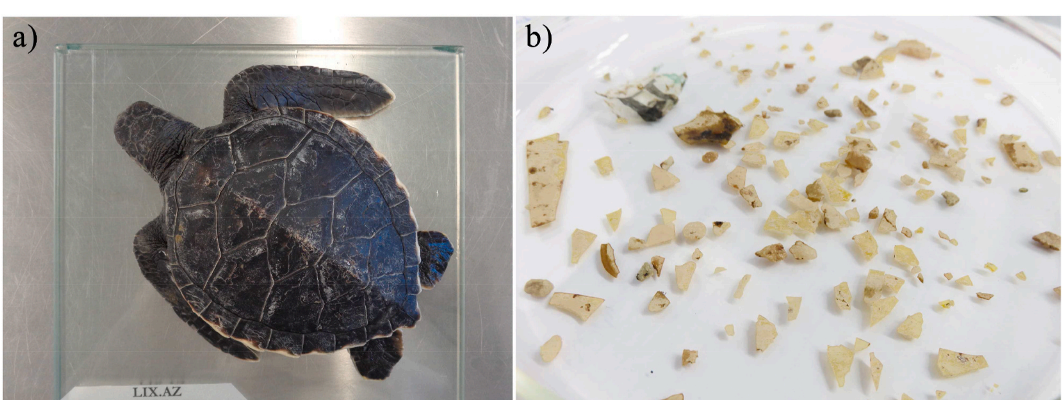

Fig. 2. (a) The smallest dead green turtle analysed in this study found in 2017 (12.1 cm CCL; 11.0 cm SCL) prepared to be necropsied. (b) The largest quantity of plastic items (n = 168; 1.526 g) recovered along the GI of a green turtle (26.2 cm CCL; 24.3 cm SCL) found dead in the Azores in 2009.

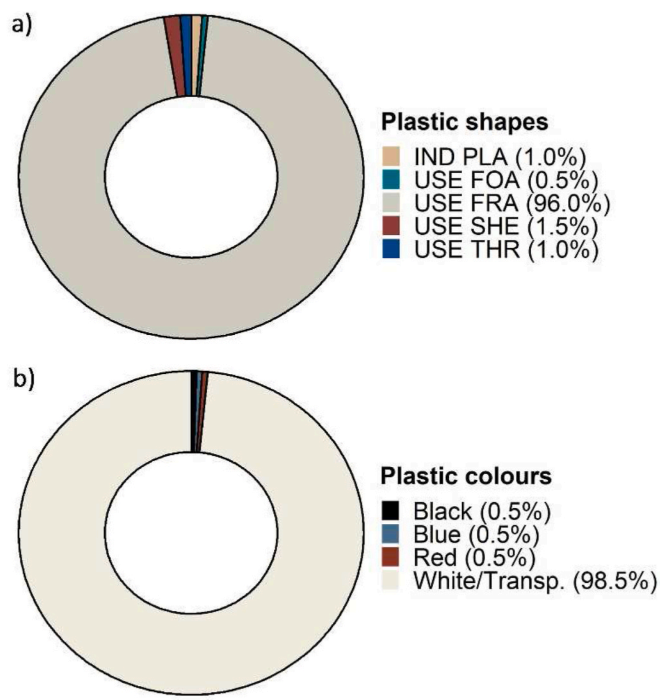

Fig. 3. Percentages of different plastic shapes (a) and colours (b) recovered from the gastrointestinal tract of juvenile green turtles in the Azores. The plastic litter shapes recovered from the GI were industrial plastics or plastic pellets (IND PLA), hard plastic fragments (USE FRA), sheet-like items or soft plastics (USE SHE), threadlike items (USE THR) and foamed plastics (USE FOA).

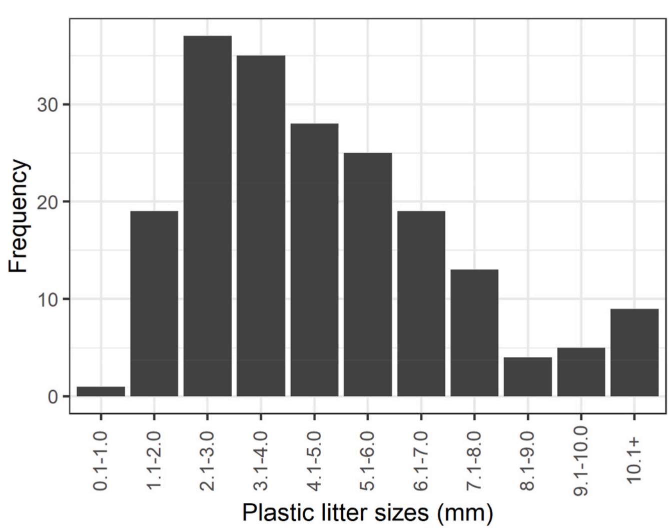

Fig. 4. Frequency of size ranges (mm) of all litter items (n = 195) recovered from the necropsied green turtles (n = 7). Overall, all anthropogenic items recovered from the GI were made of plastic.

Within each cluster, three types of hydrocarbons were detected: double bonded hydrocarbons, single bonded hydrocarbons, and saturated hydrocarbons. The chromatographic conditions did not resolve all hydrocarbons, particularly in the heavier hydrocarbon clusters occurring at higher temperatures.

3.3. Beach litter

A total of 5701 items (1–25 mm) were recovered and sorted in the laboratory. All of them were made of plastic, and the majority (92%; n = 5219) were found at Porto Pim beach on Faial Island. Overall, hard plastic fragments were the most abundant shape recorded from the surveyed beaches (USE FRA; 88%, n = 5010), followed by plastic pellets (IND PLA; 11%, n = 610), foamed plastics (USE FOA; 1%, n = 64), threadlike items (USE THR; 0.12%, n = 12) and soft plastics (USE SHE; 0.05%; n = 5). The predominant colour was white/transparent (77%; n = 4381), followed by blue (10%; n = 556), green (4%; n = 202), black (4%; n = 200) and red (3%; n = 178). Other colours represented the remaining 2% (n = 184). The PERMANOVA analysis showed no statistically significant differences between the shape of plastic items inside the turtles and the ones from the beaches (PERMANOVA: Pseudo-F = 1.419, p = 0.201; Fig. S3a). Finally, no significant differences were found between the colour of the items ingested by the turtles and the ones collected on the beaches (PERMANOVA: Pseudo-F = 1.104, p = 0.345; Fig. S3b).

4. Discussion

Analyses of stranding data in the Azores over 21 years indicate that green turtles are inhabitants of the archipelago, with about one stranding event of this species observed each year and a high frequency of interactions with plastic litter. This study further demonstrates that well-coordinated stranding networks can be an important tool for scientific research (e.g. Epperly et al., 1996; Hart et al., 2006). In the present case, it provides novel information on this sea turtle species for which little was known in this area of the Atlantic.

Our observations that larger juvenile green turtles were found stranded (from 26.8 to 46.5 cm SCL; 67% of measured turtles) along with regular observations by free divers on the coastline of the islands (Sousa, 2021), suggest that oceanic green turtles might shift to forage in shallow benthic environments in oceanic islands such as the Azores archipelago. This is in accordance with the theory proposed by Reich et al. (2007), that green turtles live within oceanic habitats of the Atlantic during their first 3–5 years, making an ontogenetic shift to neritic feeding environments when reaching sizes ranging from 25 to 35 cm SCL. Despite the fact that neritic developmental areas of sea turtles are normally described as nearshore habitats located over continental shelves (e.g. Bolten, 2003a), the present study shows that oceanic islands can also provide neritic developmental habitats. Juvenile green turtles of similar sizes originating from rookeries in the Caribbean and NW Atlantic were reported to use neritic habitats of the Canary Islands, another archipelago of the NE Atlantic located south of the Azores (Monzón-Argüello et al., 2018).

In addition, our data showed that smaller turtles (from 12 to 25 cm SCL; 33% of measured turtles) are also present in this area of the NE Atlantic. These turtles may represent a portion of neonates from Florida green turtle rookeries. However, Mansfield et al. (2021) recently excluded the hypothesis that oceanic-stage green turtles are travelers or drifters along the NASG during their first years of life, in the way that loggerhead turtles (*Caretta caretta)* do (Bolten, 2003b; Vandeperre et al., 2019). Based on the results obtained from satellite tracks of 21 green turtles (12.0–19 cm SCL), Mansfield et al. (2021) suggested that the hatchlings disperse within the Gulf Stream and then enter the Sargasso Sea. *Sargassum* areas have been previously suggested to be used by oceanic-stage of green turtles as nursery habitats in the North Atlantic (Carr and Meylan, 1980). The green turtles in Azorean waters may derive from other green turtle rookeries; genetic studies would be valuable to assess the source rookeries.

Interactions with marine litter were detected in half of the green turtles that stranded in the Azores, involving either entanglement or ingestion. Overall, entanglement was reported in 14% of the stranded turtles since 2000. Entanglement in marine litter is recognized as a major source of mortality for marine wildlife; however, most of the entanglement events are typically underreported (Claro et al., 2019) and are difficult to distinguish from bycatch. Few studies assessed this threat in green turtles (Duncan et al., 2017) and to our knowledge, no peer-reviewed study have reported entanglement in green turtles across the Atlantic so far. Our results also showed that items related to fishing activities are the main types of litter responsible for this threat, which is also the main cause for entangled propellers of the different sea-users (e.g. fishing, whale watching) operating in the region (Rodríguez et al., 2020).

At present, data on the ingestion of marine litter in green turtles is only available for western regions of the Atlantic (Bjorndal et al., 1994; Nelms et al., 2016; Lynch, 2018; Choi et al., 2021). This study provides to our knowledge, the first report of litter ingestion in green turtles across the entire eastern Atlantic area. Small white/transparent plastic fragments were the items found in highest proportion inside the GI. Indeed, not only is this similar to what was found for juvenile loggerhead turtles in the same region (Pham et al., 2017), but also other authors recently reported that high quantities of hard and white/clear plastic fragments are being ingested by post-hatchlings and oceanic phases of different species of sea turtles in the Atlantic (Eastman et al., 2020), and in the Pacific Ocean (Duncan et al., 2021). Recently, Choi et al. (2021) demonstrated differences in plastic ingestion across the different phases of the life history of green turtles in the Gulf of Mexico. Their results revealed that pelagics and recruits tend to ingest more fragments, whereas transitional and subadults found nearshore ingest more plastic sheets and threads. Sheet-like items, represented by plastic bags, wraps and packaging, have also been reported as the most abundant shape of litter ingested by green turtles in other nearshore areas of the western Atlantic (e.g. Bugoni et al., 2001; Tourinho et al., 2010; Guebert-Bartholo et al., 2011; Di Beneditto and Awabdi, 2014; González Carman et al., 2014; da Silva Mendes et al., 2015; Santos et al., 2015; Vélez-Rubio et al., 2018; Rizzi et al., 2019; Petry et al., 2021). Although soft plastic items have been considered the most dangerous because they have a higher probability of causing obstructions (Roman et al., 2020), hard plastic fragments can be sharp and, therefore, damage the GI. The fact that early life stage sea turtles are consuming this type of plastic is a matter of concern because it might affect their survival (White et al., 2018; Eastman et al., 2020).

In several locations, active selection of plastic has been proposed as the most likely pathway of plastic ingestion by green turtles (e.g. da Silva Mendes et al., 2015; Duncan et al., 2019). However, in the Azores, the characteristics of the plastics recovered from green turtles did not resemble organisms found as part of their diet (e.g. algae, fish, sea urchins or pteropods) either in terms of size or colour.

Plastic bags have been reported in the literature to be mistaken for gelatinous prey by several species of sea turtles (e.g. Mrosovsky et al., 2009; Schuyler et al., 2014) yet, we did not find any plastic bag in the green turtles although they occur in this region (e.g. Rodríguez et al., 2020). In fact, 99% of the items found in the turtles were smaller than 25 mm and no significant differences were found between shapes and colours of plastic present on the beaches and the items ingested by the turtles.

Small irregular hard fragments with white/transparent colours are the most frequent type of plastic depositing around the Azores region, similar to what was recovered from the turtles. Such shape, size and colours were previously documented as being prevalent in the Azores both on beaches (Pham et al., 2020) and at the surface (Herrera et al., 2020). Because floating marine litter and potential feeding areas of sea turtles can overlap (Pham et al., 2017), green turtles in the Azores may not select specific litter items, but ingest small plastic fragments unintentionally while feeding. Accidental ingestion of litter has been previously suggested in other populations of green turtles inhabiting the SW Atlantic (González Carman et al., 2014; Di Beneditto and Awabdi, 2014), a pathway of ingestion that appears to fit the result obtained in this study. Nevertheless, the small number of individuals analysed limits our ability to draw robust conclusions on the exact pathway of plastic ingestion in the green turtles inhabiting the NE Atlantic.

The high frequency of occurrence of plastic ingestion (86%) further confirms that green turtles are highly susceptible to feeding on plastic litter (Schuyler et al., 2012), with oceanic-stages being at higher risk in environments where marine litter is ubiquitous (Witherington et al., 2012). For example, Choi et al. (2021) found plastic ingestion in green turtles to be two orders of magnitude higher in pelagic than in subadults. Other studies from the Atlantic reported frequencies of occurrence of marine litter ingestion by green turtles between 70% and 90% (e.g. Santos et al., 2015; Colferai et al., 2017; Vélez-Rubio et al., 2018; Rizzi et al., 2019; Petry et al., 2021). In addition, and in line with previous findings in the region (e.g. Pham et al., 2020; Pereira et al., 2020), polyethylene (PE) is the main type of polymer ingested by the turtles. Polyethylene is the most commonly manufactured polymer globally and is distributed predominantly in the surface waters due to its low density compared to seawater. Therefore, the detection of polyethylene in juvenile green turtles correlates well with its high production rates and a plausible feeding behavior. The combination of these results supports the hypothesis that migratory megafauna that use the Azores as a feeding ground are exposed to anthropogenic litter dominated by plastics, even when these islands are located far away from big industries or populated cities.

5. Conclusion

This study demonstrated the Azores archipelago to be the northernmost foraging habitat of juvenile green turtles in the NE Atlantic and further revealed their vulnerability to marine litter. The turtles analysed in the present study ingested mostly small (1–25 mm) white/transparent plastic fragments. While green turtles have limited ability to adapt to the presence of plastic in the environment, the quantities of plastics in the oceans will continue to increase, as well as their fragmentation into smaller particles. This stressor could have major ecological consequences on the future well-being of green turtles in the Azores because they inhabit the edge of the NASG, an accumulation zone of floating litter. In an increasingly globalized world, studies in conservation biology should focus on understanding how the expansion and intensity of anthropogenic pressures will impact marine megafauna. Therefore, we highlight the importance of allocating the necessary resources to support well-organized stranding networks in isolated regions, but most importantly, the need to involve various stakeholders with different expertise to improve our knowledge of the threats affecting endangered cryptic species.

Author contributions

Yasmina Rodríguez: Conceptualization, Formal analysis, Investigation, Writing – original draft. Christopher Kim Pham: Funding acquisition, Supervision, Writing – review & editing. Frederic Vandeperre: Funding acquisition, Writing – review & editing. Marco R. Santos and Karen A. Bjorndal: Resources, Writing – review & editing. Laura Herrera, Hugo Parra, Ashok Deshpande: Investigation, Writing – review & editing.

Ethical approval

All appropriate ethics and other approvals were obtained for this research. Research protocols applied by the authorized animal care facility (Direção Regional dos Assuntos do Mar (DRAM)). The work was conducted under a license for the study and handling of wild marine animals (current license: LMAS-DRAM/2021/09) issued by the Direção Regional dos Assuntos do Mar and a license for the access and use of natural resources for scientific purposes (current license: 30/2021/DRCTD) issued by the Direção Regional da Ciência e Transição Digital of the Regional Government of the Azores, both issued to Frederic Vandeperre, and which are renewed annually.

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.

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Appendix A. Supplementary data

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