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Research Article

Preliminary analysis of microplastics from the main continental nesting beach of the hawksbill sea turtle (*Eretmochelys imbricata*) in Venezuela

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ABSTRACT. Microplastics are an increasing threat to marine fauna and oceanic habitats, potentially affecting sea turtle nesting beaches. Hawksbill sea turtles (*Eretmochelys imbricata*) are a Critically Endangered species with decreasing population trends. There are several hawksbill rookeries in the southern Caribbean Sea, particularly on Los Garzos Beach, the main nesting site of continental Venezuela. A preliminary physical analysis of microplastics sampled from 10 sites on Los Garzos Beach reported high numbers (94 ± 2.5 items) of 14 different materials and colors. Microplastic counts at 10 sites averaged 1504 ± 405.61 items m^{-2}, higher than other sea turtle nesting sites worldwide. There were no statistical differences in the concentration of microplastics at the sampled sites of Los Garzos Beach. However, the concentration of microplastics on this beach is higher at the midpoint of the falling tide (3520 ± 405.61 items m^{-2}) and the top of the sea turtle nesting area (3840 ± 405.61 items m^{-2}). We speculate that this debris could harm the nesting environment of hawksbill sea turtles in the region. While this study presents preliminary values for microplastics in an important hawksbill turtle nesting beach in Venezuela, it also serves as a baseline for long-term studies that can help determine the impact and sources of plastic pollution in sea turtle rookeries.

Keywords: *Eretmochelys imbricata*; microplastic pollution; nesting beach; heating capacity; toxicity; Gulf of Paria

INTRODUCTION

Microplastics are particles, pellets, pieces, or items less than 5 mm in size. It primarily results from the degradation of plastic litter and fishing nets, which can negatively impact sea biota and the marine environment in many ways, including malnutrition and altered fecundity or behavior of marine species (Ferreira et al. 2018, Nelms et al. 2018). Microplastics have recently been the subject of intense study due to an increased concern for potential impacts on the health of the oceans and marine species (Neves et al. 2015, Lindeque et al. 2020, Ugwu et al. 2021). Microplastic accumulation is more abundant in coastal areas than at sea due to ocean currents and drifts (Mizraji et al. 2017, Lopez-Monroy & Fermin 2019). It is likely impacting coastal communities that could also receive these pollutants through food ingestion (Gardon et al. 2018, Campanale et al. 2020, Yuan et al. 2022).

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Marine turtles are ecologically important and charismatic mega-fauna of the oceans; they inhabit almost all oceans but only nest over tropical and subtropical sandy beaches (Bolten 2003). These endangered species are affected by many threats, especially from anthropogenic origins like fisheries bycatch (Alio et al. 2010, Alfaro-Shigueto et al. 2011, Putman et al. 2020) and nesting habitat degradation (Maison et al. 2010, Veelenturf et al. 2020, Balladares & Barrios-Garrido 2021). An emerging concern is the impact of plastic debris, which they ingest during all their life stages (Bugoni et al. 2001, Wedemeyer-Strombel et al. 2015, Wilcox et al. 2018). Microplastics are particularly concerning as the abundance of these pollutants on sea turtle's nesting beaches has been documented in several sites, including Cyprus in the Mediterranean Sea (Duncan et al. 2018), in the northern Gulf of Mexico in Florida (Beckwith & Fuentes 2018), and in other beaches in Florida, South Carolina and Costa Rica (LeRoy & Boneillo 2019). Studies have also reported an abundance of plastics and microplastics around Ecuador's Galapagos Island of San Cristobal (Jones et al. 2021).

Early studies on marine debris impact on sea turtle grounds in the south Caribbean Sea by Barrios-Garrido et al. (2019) documented fatal ingestion of plastics, including bags and nylons, by juvenile green turtles (*Chelonia mydas*) in the Gulf of Venezuela. However, a detailed analysis of microplastics over rookeries has yet to be performed in that region. We aimed to conduct an initial physical characterization of degraded plastics on Los Garzos, the main continental nesting beach for the Critically Endangered hawksbill sea turtle (*Eretmochelys imbricata*) population in the Gulf of Paria, eastern Venezuela. Values for plastic density in this area are documented here for the first time, and we discuss possible impacts on nesting rookeries.

MATERIALS AND METHODS

Study site

Los Garzos Beach is the main continental nesting rookery for hawksbill turtles (*Eretmochelys imbricata*) in Venezuela, within the Gulf of Paria (Balladares & Quintero-Torres 2019). The central point of the beach is located using Google Earth as 10°41'61"N, 61°53'17"W. Los Garzos is part of the Peninsula of Paria National Park and near the fishing town of Macuro outside the park. The gulf is an estuarine region in the southeastern Caribbean Sea, south of the Paria Peninsula in the Sucre State, with the Orinoco River delta in the south, and located east of the western coast of Trinidad and Tobago (Fig. 1). The Gulf of Paria has an average depth of 20 m. Local currents have an anticyclone circulation, salinity ranges from 11 to 36, and sea surface temperature varies from 26 to 28ºC (Rincón et al. 2008).

The local hilly topography limits the size of this beach to approximately less than 300 m long and 75 m wide. The beach is sandy, with a low berm, and covered with supralittoral vegetation, particularly manchineel (*Hippomane mancinella* L.) and portia (*Thespesia populnea* L.) trees. These characteristics create ideal conditions for the nesting activity of hawksbill turtles.

Sample collection

We followed a sampling method similar to that of Duncan et al. (2018). With this methodology, we visited 10 sample sites on September 11, 2019, using five transect lines (transect base lines -TBL) at Los Garzos. One sample at each transect was collected from the strand line (SL), and one was collected from the turtle nesting line (NL).

The SL is the highest line of debris left from the retreating tide. There can be more than one SL depending on the tides. To standardize this, we selected the most recent SL visible as the representative SL. The turtle NL is where nests and body pits are marked over the transect. Any sample site within the turtle NL that coincided with a turtle-nesting pit was relocated 2 m to the left or right. The five transects were situated at 10% TBL (i.e. 10% between the start and the end of the beach section), 30% TBL, 50% TBL, 70% TBL, and 90% TBL (Duncan et al. 2018).

Each sand sample comprised the top 2 cm of sand in a 25×25 cm quadrat collected via a trowel. A volume of approximately 800 cm^3 (0.8 L) for each sample. Any larger pieces of plastic (>5 mm) found within the sand sample were separated to avoid post-collection fragmentation during the transport. Sand collected at each of the 10 sample sites listed above was bagged in a Ziplock bag and labeled.

Each sand sample (e.g. SL10) was reduced to 50% of its original weight for better transportation prior to labeling. During this process, the sand was thoroughly and continually mixed to prevent the separation of microplastics and sand. Additional care was taken to avoid plastic contamination, i.e. no plastic tools were used to mix the samples. Finally, a correction multiplier of 2 was used due to the subsampling process to obtain the density by weight (items kg^{-1}) and by surface (items m^{-2}). All samples weighed around 400 cm^3 (418 ± 9 cm^3 ~0.4 L) after the subsampling (Table 3).

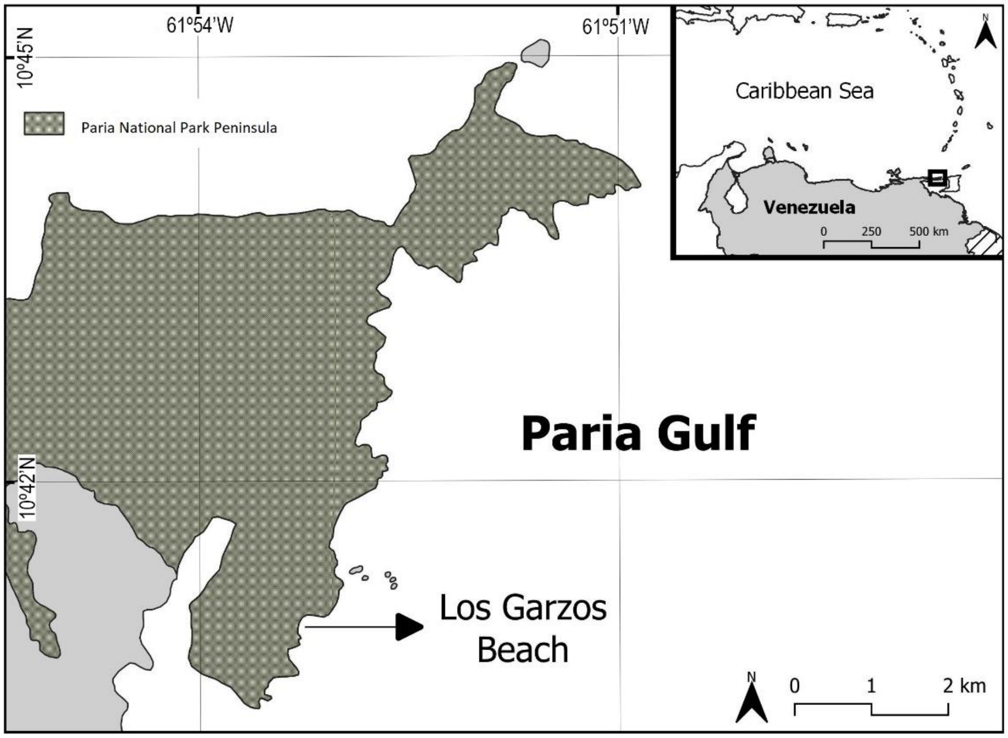

Figure 1. Los Garzos Beach. The main continental nesting rookery of the hawksbill sea turtle (*Eretmochelys imbricata*) in Venezuela.

Microplastic physical analysis

Physical analysis was performed at the Marine Biology Laboratory of the Oceanographic Institute of Venezuela (IOV, by its Spanish acronym) in Cumaná, Sucre State. Collected sand samples were dried at 60°C for 24 h on aluminum plates, then sieved via a set of metallic meshes (Fisher Scientific Company): the upper sieve was 5 mm aperture mesh, and the bottom sieve was 1 mm aperture mesh. The resulting retained material was reserved on sealed glass bottles. A completely saturated saline solution was used to extract the microplastics (Masura et al. 2015, Besley et al. 2017), prepared to dissolve 359.6 g of NaCl in 1 L of demineralized water. The solution was agitated at 600 rpm at 60°C for 8 h and then filtered through 0.45 µm Millipore paper (Darmstadt, Germany) to eliminate contaminants and impurities.

The total retained material on the 1 mm mesh was used for each sand sample to extract microplastics. This material was added to 200 mL of saturated saline solution and agitated at 600 rpm for 2 min using a magnetic stirrer mark (Magne-matic® model 15 Philadelphia, PA, USA). The sand was allowed to settle for at least 6 h. The supernatant was filtered through a 0.45 µm Millipore filter using vacuum force. Microplastic fractions remaining on the glass wall were rinsed with demineralized water (Masura et al. 2015). The extraction process described above was repeated three times. All the equipment was washed with demineralized water to avoid contamination. The filter papers were stored on clean Petri dishes for the next examination step.

For the final visual analysis, all the filter papers were examined under a stereoscopic microscope (Olympus SZ61, Japan) at 40x. All microplastics were counted systematically over 1 to 5 mm; items bigger than this were discarded (Masura et al. 2015). Morphological characterization was made according to the Standardized Size and Color (SCS) code proposed by Crawford & Quinn (2017), and the colors were registered. In order to avoid erroneous identification, we followed criteria that differentiated shells, animal parts, algae, and glass. These criteria were: 1) lack of cellular structure or organic attachment, 2) homogenous color, and 3) equally homogenous color throughout, such as fibers (Hidalgo-Ruz et al. 2012, Bosker et al. 2018). Analysis of each microplastic origin is beyond this project's scope.

Statistical analysis

A two-way analysis of variance, ANOVA, was performed on the sample microplastic content data using MATLAB. The two-way ANOVA first effect was represented by the sample locations along the lines (strand and nesting), and the second effect was represented by the two positions corresponding to the strand and the nesting lines.

RESULTS

From all 10 sample sites on Los Garzos Beach, 94 ± 2.5 microplastic items of 14 different materials were gathered (Table 1). Of all microplastics identified, 83% were fibers. The average number of items was 9.4 ± 2.5; mostly blue fibers (34), 20 transparent fibers, 16 red fibers, 9 white fragments, and film debris was the least abundant.

The sites at Los Garzos with the most microplastic items are identified in Table 2: SL50, the midpoint of the retreating line, and NL90, which is the maximum of the nesting line. The maximum densities of these items on the beach (Table 3) were 107.84 ± 12.56 and 118.23 ± 12.56 items kg^{-1} for the falling tide and nesting line, respectively. On average, microplastic density was 1504 ± 405.61 items m^{-2} for all 10 sampled sites. Overall, Los Garzos presented high concentrations of microplastic items as detailed in Table 2: SL50 is the midpoint of the retreating line, and NL90 is the highest point of the nesting line. The maximum densities of these items on the beach (Table 3) were 107.84 ± 12.56 items kg^{-1} and 118.23 ± 12.56 items kg^{-1} at the retreating and nesting line, respectively. The sites with fewer concentrations of microplastics (Table 2) were SL10, NL10, and NL50 at 18.82 ± 12.56 items kg^{-1} or 640 ± 405.61 items m^{-2}, 5.41 ± 12.56 items kg^{-1} or 160 ± 405.61 items m^{-2}, and 12.66 ± 12.56 items kg^{-1} or 480 ± 405.61 items m^{-2}, respectively (Table 3).

As only one replicate per cell existed, the interaction between the two effects (microplastic concentration among parallel and perpendicular transects) was impossible to estimate. On the other hand, the two-way ANOVA, shown in Table 4, indicates no significant effect of any factor for the analyses of the three measurements (items, items kg $m^{-1,}$ and items m^{-2}). All *F* values were associated with a probability of occurrence of more than 0.5 when the null hypothesis of no effect was true.

Table 1. Full description and item amount of microplastic found at Los Garzos Beach.

Code	Type	Amount	(%)
FBBL	Blue fibers	34	36
FBRD-WT	Red white fibers	1	1
FRRD	Red fibers	16	17
FRGN-LG	Light green fibers	3	3
FBTP	Transparent fibers	20	21
FBBL-RD	Blue red fibers	1	1
FBBK	Black fibers	1	1
FBBL-LG	Light blue fibers	1	1
FBYL	Yellow fibers	1	1
FMWT	White fragments	9	10
FMBN	Brown fragments	3	3
FLGN-LG	Light green film	1	1
ED	Red film	2	2
FLWT	White film	1	1

DISCUSSION

The present study could be the first report of this plastic pollution origin in the Paria Gulf and confirmed by the waste stream (Brooks et al. 2020). Our results indicate that the differences in concentrations of microplastics in different locations of Los Garzos Beach are not statistically different. This result should be considered with reservation because, as there is only one replicate per cell in the two-way ANOVA, we could not estimate the interaction between the two effects, parallel and perpendicular transects, to represent all sites on the beach. The graphs do not suggest any important interaction between the two effects (Fig. 2).

According to the different types of items identified at Los Garzos, a were fibers (83%) of blue, transparent, and red colors. According to Fermin (*comm. pers.*), this kind of fiber could indicate a predominantly degraded fishing net source and textiles (Lindeque et al. 2020, Cai et al. 2021). Other fragments (10%) could be from decomposed plastic containers, while films were possible debris from plastic bags and bottles (Fermin, *comm. pers.*, Jadhav et al. 2021).

Notwithstanding geographical differences and methods, a comparison of Venezuela's main hawksbill turtle's (*Eretmochelys imbricata*) nesting site at Los Garzos (Garcia-Cruz et al. 2020) to 10 loggerhead rookeries found in Florida, USA (Beckwith & Fuentes 2018) shows differences regarding microplastic concentrations. Data from Los Garzos showed higher levels of microplastics in beach sand than in Florida beaches (94 items in Los Garzos *vs*. 32 in Florida). Moreover, the density of microplastics at Los Garzos was greater (405.61 ± 160 pieces m^{-2} *vs*. 26.8 ± 16 pieces m^{-2}) at St. Joseph State Park, Florida. Although, the majority of microplastics at both locations were reported at turtle nesting sites.

Table 2. Microplastic types' distribution along Los Garzos Beach during the sampling date. SL: strand line, NL: nesting line. FBBL: blue fibers, FBRD-WT: red white fibers, FRRD: red fibers, FRGN-LG: light green fibers, FBTP: transparent fibers, FBBL-RD: blue red fibers, FBBK: black fibers, FBBL-LG: light blue fibers, FBYL: yellow fibers, FMWT: white fragments, FMBN: brown fragments, FLGN-LG: light green film, ED: red film, FLWT: white film.

	Fibers									Fragments		Films		
Site	FBBL	FBRD-	FRRD	FRGB-LG	FBTP	FBBL-RD	FBBK	FBBL-LG	FBYL	FMWT	FMBN	FLGN-LG	FLRED	FLWT
SL10	3									1				
SL30	6													
SL50	2	1	15	3							1			
SL70	2						1			1		1		
SL90			1		8	1					1			
NL10					1									
NL30					5									
NL50					2			1						
NL70	1				4				1	7				
NL90	20										1		2	1
Total	34	1	16	3	20	1	1	1	1	9	3	1	2	1

Table 3. Weight and densities of the sites on the beach. SL: strand line, NL: nesting line.

Site	Sand dry weight (cm^3)	Items	Density by weight×2 (items kg^{-1})	Density by surface×2 (items m^{-2})
SL10	425	4	18.82	640
SL30	427	6	28.10	960
SL50	408	22	107.84	3520
SL70	440	5	22.72	800
SL90	401	11	54.86	1760
NL10	370	1	5.40	160
NL30	410	5	24.39	800
NL50	474	3	12.65	480
NL70	420	13	61.90	2080
NL90	406	24	118.22	3840

Unpublished data of a sea turtle conservation project in the area conducting biennial beach cleanings in Los Garzos from 2003 until 2018 revealed an average of 413.8 ± 90.1 kg of common plastic bottles recovered site at each cleaning (Venezuela Environment Ministry unpublished). Commercial labels of debris collected show that the source of these plastics was from Trinidad and Tobago, likely transported by the local anticyclone currents (Rincón et al. 2008). This preliminary study could be used as a baseline for microplastic presence and density in an important nesting beach in Venezuela.

Turra et al. (2014) have pointed out that debris must be studied on a 3D quantification protocol to extrapolate contamination levels beyond the surface. However, microplastics are generally reduced with increasing depth, especially in the nesting pit (Duncan et al. 2018). For sea turtle nesting beaches, pollutant analyses could include the microplastics' chemical composition and resulting toxicity (Beckwith & Fuentes 2018, Duncan et al. 2018). Elucidating the impact of these pollutants on nesting sea turtles requires consideration of other physical aspects, including temperature, which is a key factor for the nesting environment. The heating capacity of these materials could increase the overall temperature of the nest due to the greater specific heat of plastics compared to sand and increased heat capacity from darker items (Andrady 2011, LeRoy & Boneillo 2019), even at 60 cm depth (Zhang et al. 2022). In the present work, such

Table 4. Two-way ANOVA for the three variables in Table 3. F: ratio between the two variances.

Source of variation	Sum of squares	Degrees of freedom	Mean sum of squares	F
		Number of items		
Lines	276.4	4	69.1	0.92 ($P > 0.5$)
Distances along lines	0.4	1	0.4	0.01 ($P > 0.9$)
Error	301.6	4	75.4	
Total	578.4			
		Items kg^{-1}		
Lines	6795.3	4	1698.8	0.92 ($P > 0.5$)
Distances along lines	9.6	1	9.5	0.01 ($P > 0.9$)
Error	7392.4	4	1848.0	
Total	14197.3	9		
		Items m^{-2}		
Lines	7075840	4	1768960	0.92 ($P > 0.5$)
Distances along lines	10240	1	10240	0.01 ($P > 0.9$)
Error	7720960	4	193024	
Total	14807040			

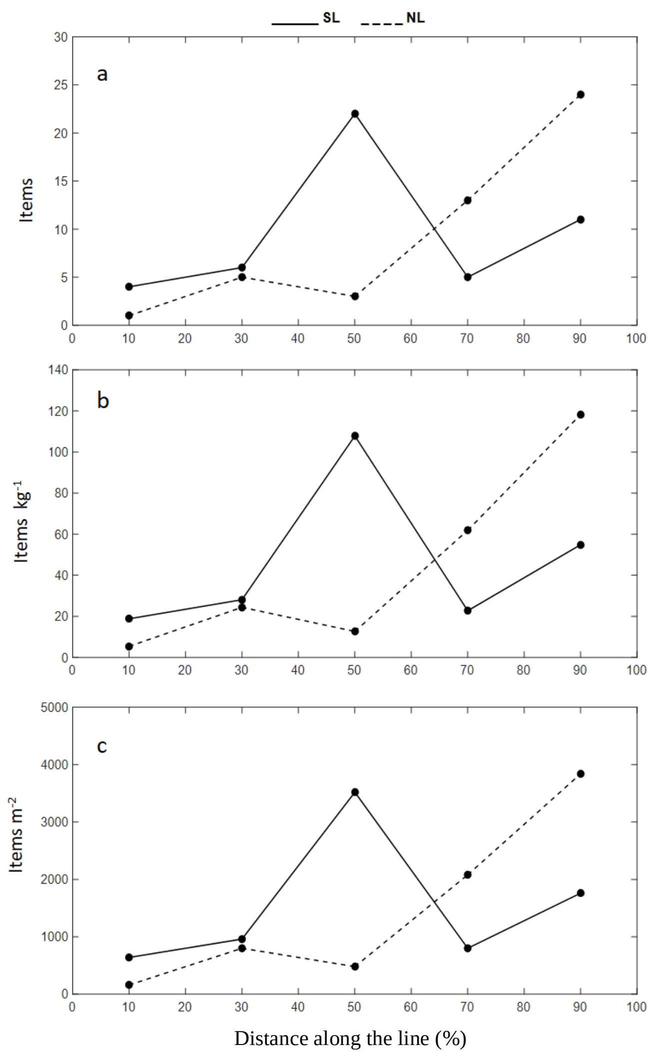

Figure 2. Graphs of the three variables in Table 2. a) Items per distance along the line, b) items kg^{-1} per distance along the line, c) items m^{-2} per distance along the line. SL: strand line, NL: nesting line.

factors were not evaluated. However, this is a critical area of future research to understand better the physical and chemical impacts of microplastic contamination of sea turtle nesting sites.

Our study is a baseline for microplastic assessment in the Southern Caribbean. However, future assessments should include other important nesting beaches of the region like Paria North and Margarita Island, key areas for leatherbacks (*Dermochelys coriacea*), Aves Island, an important green turtle (*Chelonia mydas*) site, and Los Roques Archipelago, another important location for hawksbills. Further studies could include increased sampling and replicates, Spatio-temporal factors, analysis of the chemical composition of the items, and possible ingestion by hatchlings at the beach.

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