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Plastic ingestion in giant tortoises: An example of a novel anthropogenic impact for Galapagos wildlife☆

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A R T I C L E I N F O

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A B S T R A C T

The human population of Galapagos has rapidly increased in the last decades accelerating the anthropogenic pressures on the archipelago's natural resources. The growing human footprint, including inadequate management of garbage, may lead to conservation conflicts. Here, we assessed the ingestion of debris by Western Santa Cruz giant tortoises (*Chelonoidis porteri*) within human-modified and protected areas. Additionally, we characterized environmental debris and quantified tortoise abundance together with tortoise fecal samples. We processed a total of 6629 fecal samples along a gradient of anthropogenic disturbance based on human debris presence. We found 590 pieces of debris in samples within human-modified areas (mean of 3.97 items/kg of feces) and only two pieces in the protected area (mean of 0.08 items/kg of feces). Plastic waste was the predominant category in feces within the anthropic area (86.3%; n = 511), followed by cloth, metal, paper, synthetic rubber, construction materials, and glass. On average, the proportion of plastic was higher in feces (84%) than it was in environmental debris (67%), denoting that plastics are more readily ingested than other types of debris. We also found that green, white, and light blue plastics were consumed more often than their prevalence in the environment, suggesting color discrimination. Tortoise abundance was higher in the protected area when compared to the human-modified area; however, recapture rates were higher in anthropized landscapes which increases tortoise exposure to plastics and other human associated threats. Our results indicate that plastics are frequently consumed by tortoises in the polluted anthropic areas of western Santa Cruz, but scarce in protected areas. More research is needed to understand the negative impacts associated with plastics for Galapagos terrestrial species. We encourage local stakeholders to implement current policies limiting expansion of urban areas, plastic use, and improving waste management systems to minimize threats to human and animal health.

1. Introduction

The Anthropocene is the current geological epoch characterized by the growing human footprint leading to global impacts that affect planetary health (Crutzen, 2002; Keys et al., 2019; Zalasiewicz et al., 2020). Earth is increasingly threatened by climate change, the extinction of species, land-use changes, the exploitation of organisms, pollution, and invasive alien species (Fordham and Brook, 2010; Deem et al., 2019; Baho et al., 2021). Among these challenges, anthropogenic pollution is one of the greatest threats and is leading to the loss of biodiversity and harming animal, human, and environmental health (Myers et al., 2013). Plastic is among the most prevalent global pollutants (Díaz et al., 2019; Marques et al., 2021) and it is estimated that the input of plastics into ecosystems will triple within 20 years unless immediate action is taken to reduce their production and limit their use (da Costa, 2021).

The ubiquity of plastics represents a major threat to the health of wildlife species, as plastics are often ingested leading to gastrointestinal impactions and obstructions, injuries, and can also result in

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entanglement (Gall & Thompson, 2015; Lima et al., 2019; Jones et al., 2021; Thrift et al., 2022). Furthermore, chemicals within plastic may cause endocrine disruption, and more recently microplastic particles have been shown to act as vectors of invasive species, infectious diseases, and even antimicrobial resistance (Bhandari et al., 2015; Pham et al., 2021; Leslie et al., 2022). Recent studies have documented plastic waste in at least 1565 species worldwide, of which 277 are terrestrial and freshwater and the remainder belong to marine ecosystems (Santos et al., 2021). Terrestrial species might be underrepresented due to a lack of studies for land compared to marine ecosystems (Staffieri et al., 2019; Blettler & Mitchell, 2021; Kutralam-Muniasamy et al., 2021; Gamarra-Toledo et al., 2023).

The Galapagos Islands are a global conservation icon, and they are one of the most emblematic places for unique marine and terrestrial flora and fauna (Reck, 2014). However, the archipelago is not exempt from anthropogenic environmental degradation, including habitat loss, pollution, invasive species, and climate change (Sachs & Ladd, 2011; Toral-Granda et al., 2017; Alfaro-Núñez et al., 2021). Plastic pollution has recently been documented in the Galapagos marine reserve, with up to 52% of the analyzed marine invertebrates ingesting microplastics (Jones et al., 2021). Additional impacts have been observed, including flightless cormorants (*Phalacrocorax harrisi*) building their nest with plastic bags, and sea turtles and sea lions mistaking plastics for food or getting trapped or entangled (Zambrano-Monserrate & Ruano, 2020; Jones et al., 2021; Muñoz-Pérez et al., 2023). Despite an increasing amount of garbage can be observed on land for all five human-populated islands, its magnitude and potential impact to wildlife health has not been documented. The impact of plastics in terrestrial Galapagos species was described by Harvey et al. (2021), reporting a mortality of up to 18% in Darwin's finches due to anthropogenic debris used to build their nests, and leading to hatchling entanglement and strangulation. Very recently, a comprehensive assessment of Galapagos plastic pollution along the coastline described microplastic abundance ranging from 0.003 to 2.87 items/m^2 in all five marine reserve bioregions (Muñoz-Pérez et al., 2023). Through citizen science, this research documented plastic exposure in 52 Galapagos marine and terrestrial species and identified Santa Cruz tortoises (*Chelonoidis* spp.), green sea turtles (*Chelonia mydas*), marine iguanas (*Amblyrhynchus cristatus*), black-striped salemas (*Xenocys jessiae*), and Galápagos sea lions (*Zalophus wollebaeki*) at the highest risk of harm due to the ingestion of plastics (Muñoz-Pérez et al., 2023). Modelling approaches have identified continental inputs as a major source of incoming plastic contamination to Galapagos coasts, mostly from southern Ecuador and northern Peru (Van Sebille et al., 2019). Oceanic currents and the anthropic pressure of use of the beaches were the main factors that determined the level of macroplastics and the diversity of items (Sanchez-Garcia & Sanz-Lazaro, 2023).

Galapagos tortoises (*Chelonoidis* spp.) are one of most emblematic species of the archipelago and can act as bio sentinels of ecosystem health (Nieto-Claudin et al., 2022). Giant tortoises play key roles maintaining healthy ecosystems in the archipelago as well as supporting local economies based on tourism (Blake et al., 2021; Frazier, 2021). Santa Cruz is the most human-populated island and represents 60.2% (17,233 inhabitants) of the entire Galapagos population (INEC, 2023). The town of Puerto Ayora is the main touristic hub of the archipelago, and the influx of national and international tourism highly contributes to resource demand and the generation of garbage. Additionally, this island supports two critically endangered giant tortoise species (*Chelonoidis porteri* and *Chelonoidis donfaustoi*) (Cayot et al., 2017a,b). The Western Santa Cruz tortoise (*C. porteri)* has an estimated population of more than 6000 individuals, and it is the species that overlaps the most with anthropogenically disturbed areas (Nieto-Claudin et al., 2021; Pike et al., 2021). During their seasonal migrations driven by vegetation dynamics and food availability, tortoises in Santa Cruz leave the protection of the National Park areas and enter private land (i.e., agricultural, livestock, urban, and rural areas), where they may be exposed to numerous threats including vehicle strikes, introduced species, and exposure to agrochemicals and resistant bacteria (Flanagan, 2021; Nieto-Claudin et al., 2021; Nieto-Claudin et al., unpublished data). This exposure was exacerbated in 2009, when 70 ha of the Galapagos National Park were transferred to the Municipality of Puerto Ayora to increase the urban area of the largest city in the Galapagos (a neighborhood currently known as "El Mirador"; Bonilla et al., 2020).

In recent years, we have observed increased local plastic pollution in the environment and encountered plastics in tortoise feces within anthropic areas, prompting the current study. The lack of data on free-living giant tortoises' exposure to, and potential ingestion of debris, led us to design a study to describe and characterize anthropogenic waste in the feces of *C. porteri* within areas with varying levels of anthropogenic disturbance. Additionally, we characterized the environmental debris available for tortoises to ingest and quantified tortoise abundance and recorded any signs of injury or disease at the study areas. Our hypothesis was that giant tortoises inhabiting polluted environments would be ingesting greater amounts of debris than those from protected areas.

2. Materials and methods

2.1. Study site

The current study was conducted in Santa Cruz Island, the most human-populated of the Galapagos Islands and located in the center of the archipelago (S00.704296°, W090.331475°). We collected data from two areas of Santa Cruz Island: (1) a protected area within the National Park called "La Torta" and (2) a human-modified area that included farmland, industrial, and urban sites (Fig. 1).

We sampled along a 4.6 km transect within "La Torta" that extends from 65 m to 130 m above sea level. La Torta (S00.719907, W090.360582) is a protected area with little to no human impact and is regularly monitored by the Galapagos National Park rangers to assess tortoise nesting sites. Hereafter, we will refer to this transect as the "National Park transect" (NPT). No human activities, including tourism, are permitted within the protected area, except for hunting of invasive species (e.g., wild boars, goats) and scientific research.

The eastern end of the National Park transect was followed by the second transect of 7.7 km which traversed several zones under anthropogenic disturbance with an elevation gradient from 26 m to 130 m above sea level. Hereafter, we will refer to this transect as the "human-modified transect" (HMT). This transect was subdivided according to its predominant land-use type, into five categories (Fig. 1): (1) agriculture: crop and livestock farms including the presence of houses with no sewage systems; (2) rural road: recently opened unpaved road mainly used to connect the agriculture and industrial areas; (3) industrial: locally called "Artisanal Park" where environmental and acoustic pollution exists from industrial and artisanal activities including carpentry, ship building, block construction, and automobile mechanics; (4) main road: paved Baltra highway which is the only access to the city of Puerto Ayora; and (5) urban: highly populated neighborhood of "El Mirador" that was part of the National Park area until 2009 when it was added to the town of Puerto Ayora.

2.2. Sampling design and sample collection

2.2.1. Quantification of debris within tortoise feces

Fecal samples from *C. porteri* were collected during weekly surveys (n = 34) in the human-modified transect (HMT) and monthly surveys (n = 8) in the National Park transect (NPT), from April to November 2021. On each survey, we walked the whole transect during morning hours (6h30 to 13h00), from northwest to southeast in the HMT and from southwest to northeast in the NPT. Fecal samples were collected by hand using sterile gloves. The number of feces was recorded per transect, and all samples from the same transect were preserved together in labeled

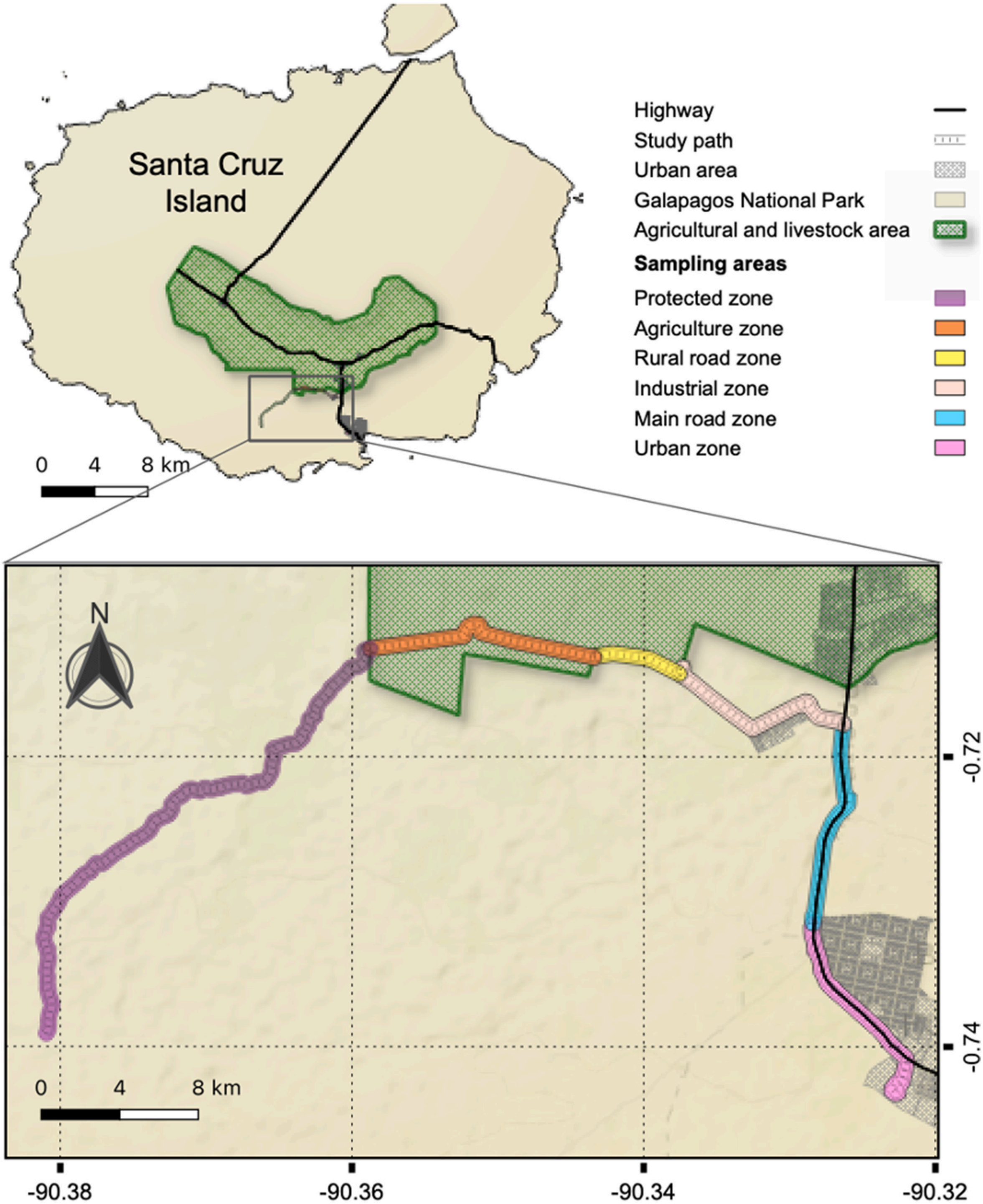

Fig. 1. Sampling locations of giant tortoise' plastic ingestion study in Santa Cruz Island according to its predominant land-use type: protected, agriculture, rural road, industrial, main road, and urban area.

Ziploc bags until analysis. In the HMT, we removed all feces from the trail one week before starting the study. Consequently, only feces expelled since the previous survey were collected. We collected a total of 1039 tortoise feces (26.1 kg) from 8 surveys within the NPT, and 5590 feces (156.1 kg) from 34 surveys within the HMT.

Within 6 h of collection, fecal samples were transported to the laboratories of the Charles Darwin Research Station (CDRS) and frozen (−20 °C) for 24–48 h to kill invertebrates. Following the thawing of the feces, all samples were dehydrated for 7 days at a constant temperature of 45 °C by using an artisanal plant dryer made with nine lamps (200 WATT). Dry samples from each transect were weighed with an analytical balance (BOECO, BWL51), followed by a close examination using gloves and tweezers to manually disaggregate each sample and identify anthropogenic residues. We identified and quantified all items that were observable to the naked eye (≥1 mm). In addition, we used a magnifying glass and a stereoscope to verify their characteristics. For every item, we recorded size (length and width), color, state of degradation (ranging from 0 to 3, 0 no degradation, 1 slightly degraded, 2 visible degradations with partial loss of color/integrity, and 3 highly degraded with complete loss of integrity and fragments falling apart during manipulation), and type of material using the categories shown in Table 1. The same researcher (KR) conducted all measurements and classifications to avoid observer bias. Plastics were further classified into microplastics (≤5 mm) and macroplastics (>5 mm) as described in the literature (Jones et al., 2021). Those plastic particles smaller than 1 mm were recorded as observed but not counted. We collected all samples under the Galapagos National Park annual research permit PC-17-21,

2.2.2. Characterization of environmental debris availability

We quantified the amount of debris available for tortoise ingestion in both sampling areas and within both seasons (dry and wet). This work was performed at separate times from the tortoise counts and fecal surveys. To capture the variation of debris along the length of each transect, 20 m^2 quadrants were placed at random locations along the length of each transect ($n = 14$ locations along the NPT and 18 locations along the HMT). The surveys were conducted during the wet season (May) and the dry season (September) for a total of 28 quadrants in the NPT and 38 quadrants in the HMT. Anthropogenic debris was quantified and characterized per quadrant, and a photo with a 150 cm flexible measuring tape was taken so debris items could be measured. To avoid potential bias by affecting debris availability in the study area, all items were returned to the collection sites until the completion of the study. Items were characterized and classified using the criteria shown in Table 1 to allow comparison with fecal samples.

2.2.3. Giant tortoise distribution and abundance

We estimated tortoise population relative abundance in both transects using mark-recapture surveys concurrent with the fecal sample collection. All tortoises that were observed from the transect (≤10 m distance) were included. Tortoises encountered for the first time were marked with a unique number painted with nail polish on the dorsal-caudal scutes of the carapace. All marked tortoises found on future surveys were considered as "recaptured". On each survey, we recorded every individual tortoise found. As with the fecal surveys, mark-recapture surveys in the HMT were weekly while NPT were monthly, from April to November 2021. A GPS location for each tortoise was recorded as well as curved carapace length (CCL), dorsal photo, sex (male, female, undetermined) and age (adult, subadult, or juvenile; based on tail length and CCL as described in Nieto-Claudin et al., 2021), activity (i.e., sleeping, resting, walking, eating, copulating, drinking), and any signs of injury or disease (based on a visual exam). Mark-recapture surveys were used to correlate tortoise presence with the abundance of feces and their migratory patterns.

Table 1
Debris fragments found within Western Santa Cruz giant tortoise (*Chelonoidis porteri*) feces and in the environment of the human-modified transect (HMT), classified by type of material and expressed in percentage (%).

Type of material	% in feces from HMT	% in the environment from HMT	Description of items (entire or fragments) included by type of material *All of them found in both the feces and the environment
Cloth	8.4	8	clothing, thread, string, sandpaper cloth, shoes, wiper or cleaning cloth
Construction material	0.5	2.2	roof and floor tile fragments
Glass	0.3	5.6	beer and soft drink bottles
Metal	2	5.4	wrapper, wire, metal mesh, bottle cap
Paper/cardboard	1.7	9.7	newspaper, card box
Plastic	86.3	65.4	bags, plastic wraps, containers, bottles, ropes, foam, scotch tape, labels, nylon, tube, broom sow, dish, disposable face mask
Synthetic rubber	0.7	3.7	tube, bracelet, balloon

2.3. Statistical analysis

To compare the number of items of debris per kg of feces between the National Park vs the human-modified area and by season and land-use type we used linear mixed effects models to account for repeated measures using the 'lme4' package (Bates et al., 2015). For mixed models, we included survey id as a random factor to account the multiple observations per survey. To compare differences in the density of environmental debris between habitats, season, and land-use types we used linear models using the 'stats' package (R Core Team, 2021). To compare differences in the number of tortoises per km between habitats and land-use types we used linear mixed effects models, and for differences in recapture ratios among land-use type and tortoise sizes (CCL) we used a binomial general linear mixed model also from the 'lme4' package. Any post-hoc comparisons were done with the 'emmeans' package (Lenth, 2020). We also obtained descriptive statistics of debris characteristics and of tortoise capture and recapture events clustered by sampling areas, land-use type, mean elevation per transects, sexes, and ages to identify if any age or sex group were most affected by debris ingestion than others and to correlate with tortoise movement patterns. Means are given with standard error or 95% confidence intervals for the estimate. All statistical analyses were undertaken in RStudio Version 4.1.1 (R Core Team, 2021; RStudio Team, 2019).

3. Results

3.1. Quantification of debris within tortoise feces and the environment

Only two fragments of debris were found in tortoise feces within the National Park. In contrast, a total of 590 pieces of debris were found in tortoise feces within the human-modified area (Fig. 2). This resulted in an estimated mean of 0.08 ± 0.89 items/kg of feces in the National Park, which was significantly less ($t_{(32)} = -3.84$, $p < 0.001$) than the 3.97 ± 0.48 items/kg of feces in the human-modified area (Fig. 3). A similar pattern was found for debris collected in the environment: no debris was found in the National Park, whereas 462 items of debris were found in the human-modified area. This resulted in at least one item of debris likely to be encountered every 2 m (mean density of 0.64 ± 0.08 items/m^2) within the human modified area, which was significantly greater ($F_{(62)} = -26.57$, $p < 0.001$) than the likelihood of encountering debris in the National Park (Fig. 2). We found no seasonal effects on the mean density of debris both in feces ($t_{(11)} = 0.01$, $p = 0.99$) or in the environment ($F_{(62)} = 1.55$, $p = 0.21$).

Amongst land-use types within the human-modified area, the amount of debris in the environment was variable, with some land-use types consistently having more debris than others. The land-use type with the highest mean density of environmental debris was the industrial area (1.11 items/m^2 95% CI 0.55–1.66), followed by the main road (0.98 items/m^2 95% CI 0.43–1.53), agriculture (0.42 items/m^2 95% CI -0.13-0.98), urban (0.34 items/m^2 95% CI -0.21-0.89), and rural road (0.08 items/m^2 95% CI -0.71 - 0.86). There were also differences in the amount of debris found in tortoise feces amongst land-use types. We found the greatest concentration of debris in tortoise feces along the main road (10.55 items/kg 95% CI 5.75–15.36), followed by urban (5.83 items/kg 95% CI 1.02–10.64), industrial area (3.28 items/kg 95% CI -1.53–8.09), agricultural (2.31 items/kg 95% CI -2.50-7.12), and none found along the rural road. The feces within the main road also had significantly ($p < 0.05$) more debris items/kg than all other land-use types except the urban area. Most debris items in feces were fragments (547 items) rather than complete (45 items), with an average weight of 0.32 ± 0.03 g, length of 9.77 ± 0.54 cm, and width of 2.56 ± 0.18 cm.

All debris types present in the environment could also be found in tortoise feces including glass (Table 2). In both the environment and in

Fig. 2. A Galapagos giant tortoise (*Chelonoidis porteri*) ingesting plastics in the human-modified area of Santa Cruz Island (A); fecal samples collected in the human-modified area containing plastic debris (B); and examples of debris found within tortoise feces, including a sanitary face mask (C).

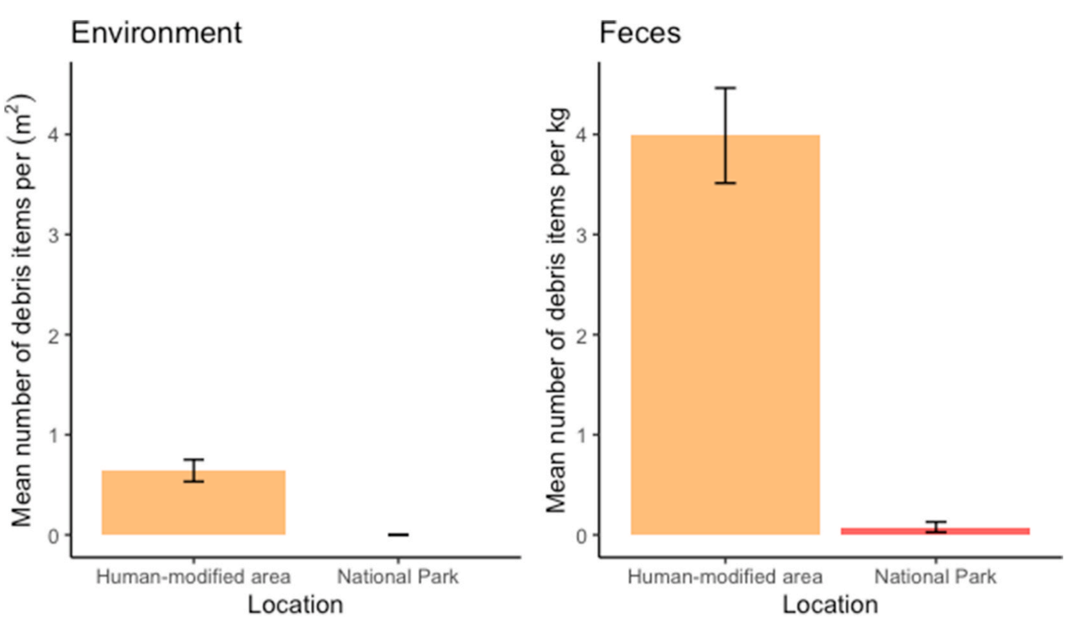

Fig. 3. Comparison of the mean number of debris items found within the environment in both the National Park, and the human-modified habitat (left) as well as the within tortoise feces (right). On average, debris was much more prevalent in human-modified habitat and frequently found in both tortoise feces and the environment.

Table 2
Giant tortoise captures, recaptures, abundance, and average size (curved carapace length- CCL-with standard error) clustered by study area and land-use type for the Galapagos tortoise' plastic ingestion study in Santa Cruz Island.

Transect	Land-Use Type	Elevation (m)	Transect length (km)	N captures	N recaptures	Tortoise Abundance		
						# per km/ survey	Average size ± se (CCL)	Size range (CCL)
National Park Transect (NPT)	Protected	65–130	4.6	153	80	7.1	76.4 ± 2.5	11–152
Human-Modified Transect (HMT)	Agricultural	130–142	1.9	123	234	6	99.2 ± 2.2	51–154
	Rural road	142	0.72	7	3	1.4	94.2 ± 8.9	60–126
	Industrial	142–177	1.6	20	152	0.8	84.5 ± 4.1	54–108
	Main road	117–73	1.6	16	70	4.5	65.7 ± 3.6	43–100
	Urban	73–26	1.8	42	104	4.4	72.2 ± 2.9	35–125

tortoise feces, plastics were the dominant type of debris (Fig. 4). However, on average plastics were a larger proportion of debris items found in feces (84% ± 3% of debris items per survey), than they were in the environment (67% ± 4% of debris items per quadrat).

Plastics in the environment belonged to a wide range of colors (Fig. 4). The predominant colored plastics found in the environment were transparent (30%), white (14%), green (11%), black (10%), and dark blue (10%; Fig. 4). In comparison, however, some colors were more frequently ingested by tortoises than others, such as white (23%), transparent (19%), green (18%), and light blue (12%), while darker colors such as grey, dark blue, and silver were less frequently consumed, especially in comparison to their relative abundance in the environment (Fig. 5).

3.1.1. Giant tortoise distribution and abundance

We recorded a total of 233 tortoises in 8 transect surveys within the NPT and 771 tortoises in 34 transect surveys within the HMT. Tortoise abundance was significantly ($t_{(33)} = 9.31$, $p < 0.01$) higher in the National Park with an estimated mean of 6.3 (95% CI 5.6–7.1) tortoises/km per survey versus 3.0 (95% CI 2.1–3.8) tortoises/km in the anthropic area. Within the human-modified areas, we found that on average tortoise abundance was generally low within the urban (2.6 tortoises/km 95% CI 0.8–4.4), rural road (2.4 tortoises/km 95% CI -0.05–4.9), main road (1.7 tortoises/km 95% CI -0.08–3.6), and industrial area (3.3 tortoises/km 95% CI 1.5–5.1). The agricultural area however had significantly more tortoises on average (5.5 tortoises/km 3.8–7.3, $p<0.05$) than the other land-use types within the human-modified area but was not significantly less than tortoise abundance in the National Park ($p = 0.34$).

Tortoise recapture events were common in both the National Park (80 recapture events) and the human-modified transect (563 recapture events) (Fig. 5). In the NPT, 25.5% of marked tortoises were observed at least two times, compared to 73.6% in the HMT. In the NPT, 7 tortoises (4.6%) were observed 4 or more consecutive months and one juvenile was observed 7 out of 8 survey events. In the HMT, 24 tortoises (11.5%) were observed for 4 or more consecutive months (most of them within the agricultural area) with three animals (1.4%) observed at least one time every month during the whole sampling period. Six animals (2.9%) were recaptured more than 15 times and two (0.9%) more than 20 times.

There were differences in the probability of tortoises being recaptured among land-use types (Fig. 6). The industrial zone had the highest tortoise recapture ratio on average (mean odds ratio = 6.5 95% CI 2.6–16.3), compared to other land use types (Fig. 6). In the protected area the recapture to capture ratio was 0.5. Overall, smaller tortoises were more likely to be recaptured ($z = -5.56$, $p < 0.001$). Only one adult male tortoise initially captured in the protected area was recaptured in the anthropic area (agricultural zone). The proportion of sex (male, female, undetermined) and age (adult, subadult, or juvenile) differed across elevation (bigger and more mature animals at higher elevations) and land-use type.

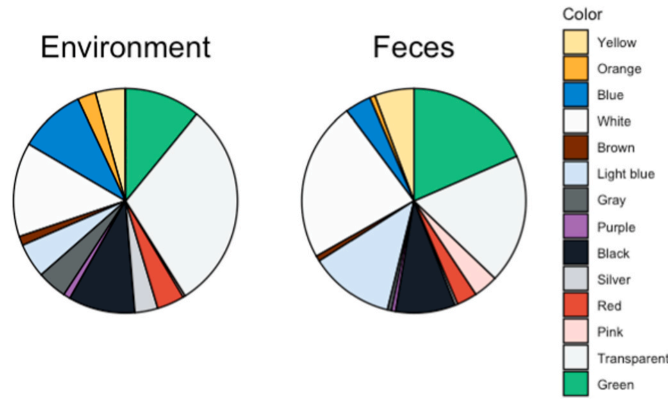

Fig. 5. Comparison of the percentage of plastic debris grouped by color found in tortoise feces and in the environment within the human modified areas of Santa Cruz Island.

During the course of this study, additional tortoise health threats were observed within both protected and anthropized areas. These included an adult male tortoise (CCL = 152) entangled by a rope within a pool in the agriculture area, a subadult female (CCL = 71.2) recaptured six-times in the industrial area was observed the last time with an injury of the cranial carapace, most likely due to a car impact, and a dead subadult tortoise with multiple fractures of the carapace found by the edge of the road in the agriculture area (Supplementary materials; Fig. 7). In addition, 13% (20/153; 95% CI 0.09–0.19) of the tortoises from the protected area and 7% (14/208; 95% CI 0.04–0.11) from the anthropized area presented lesions on the edges of the carapace consistent with dog attacks.

4. Discussion

In this study we show that a critically endangered species of giant tortoise, *C. porteri*, frequently ingests plastics in anthropic areas of one of

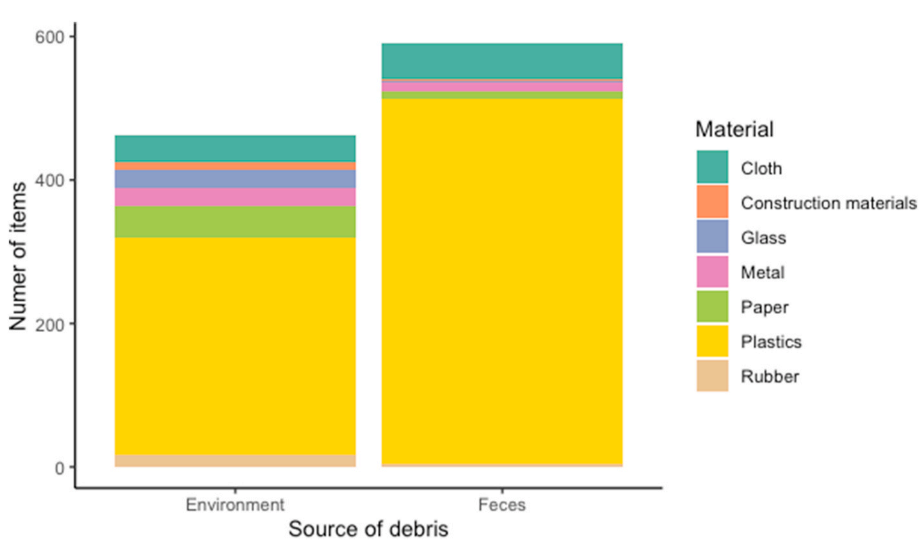

Fig. 4. Comparison of the total number of each type of debris found within the environment to those found within Galapagos giant tortoises' feces in the human-modified areas on Santa Cruz Island. Plastic was the dominant type of debris found in both locations.

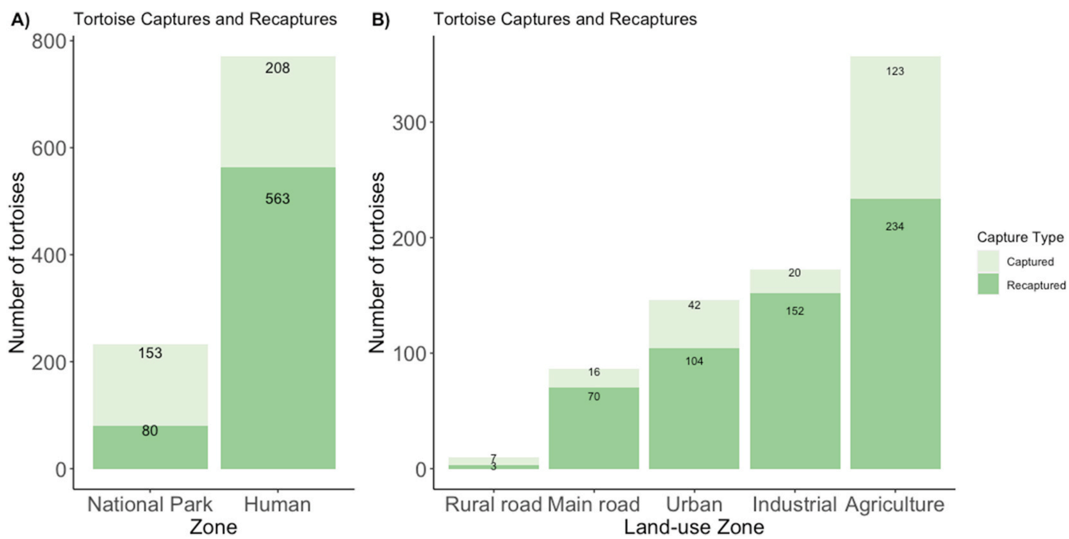

Fig. 6. Giant tortoise capture and recapture (captured two or more times) rates clustered by study area (A-total numbers for 8 surveys in the National Park area and 34 surveys in the human-modified area) and land-use type within the anthropogenic area (B).

the most isolated and pristine archipelagos in the world, the Galapagos. In contrast, plastics were almost absent in tortoises' feces from the protected National Park area. This is the first comprehensive study on plastic ingestions rates in Galapagos giant tortoises. In the human-modified area, you are likely to encounter a piece of rubbish every 2 m on average which may explain why it was frequently ingested by tortoises in this habitat. Our findings suggest that these tortoises ingest plastics when present in their environment, underscoring the need for strict plastic use regulations in the Galapagos Islands and improved waste management systems.

Although there is no information on the effect of macro (prior to this study) or micro-plastics on giant tortoise health, studies in other animals prove adverse effects for both types of plastics. Endocrine disruptor chemicals (EDCs) have been described as omnipresent chemicals that can be found within all types of plastic materials (Encarnação et al., 2019). Of mounting concern in the plastic associated EDC category is Bisphenol A (BPA), the most widespread EDC in marine and terrestrial habitats, negatively influencing the health and reproductive fitness of animals and humans (Bhandari et al., 2015; Deem & Holliday, 2022). Recent studies in shearwaters (*Ardenna carneipes*) have shown a novel plastic-induced fibrotic disease characterized by widespread scar tissue formation in the digestive tract (Charlton-Howard et al., 2023). Our results indicate that the negative effects of macro and micro-plastic need to be assessed in giant tortoises given the high frequency of plastic ingestion.

The negative effects of plastics on the health and reproductive physiology of reptiles may be greatest for critically endangered species such as the giant Galapagos tortoises (Tubbs & McDonough, 2018). Juveniles remain for longer periods in low elevation anthropic areas and near the town of Puerto Ayora, Santa Cruz Island. Therefore, plastics in the diet of immature individuals may create a greater risk to their fitness and consequently for the long-term survival of this species. Recapture rates observed in the current study supports that tortoises do not only migrate through human-modified areas such as farmland but can remain for long periods of time (weeks to months) in highly altered and polluted environments where they are exposed to plastic ingestion, as described in recent studies (Pike et al., 2021, 2022a).

Our results show that tortoises might be color-selective when feeding on debris. It has been described that chelonians have good vision for red, yellow, and orange wavelengths, which may partially explain tortoise attraction to some colorful foods (Boyer and Innis, 2019). Another study conducted in captive giant tortoises used color discrimination trials with different colored targets, and tortoises were able to distinguish and memorize it (Gutnick et al., 2020). In the current study, we observed tortoises frequently ingesting green, white, and light blue plastic items which may indicate that giant tortoises can discriminate warm color plastics from fruits and flowers they eat. In contrast, tortoises ate less dark blue or grey items, especially relative to their availability in the environment. It is unclear whether tortoises are choosing to consume plastic items, however it is possible that tortoises may have consumed more green or white items by mistaking them for vegetation. To further investigate if tortoises have a preference for foods with certain colors, additional research should be conducted on food color preferences. Although some plastic items including bags with handles and straws were prohibited in Galapagos in 2015, plastics continue to be broadly available in the archipelago and a complete ban of the use of plastics seems unlikely. Color preference trials may inform local policy makers whether restricting the use of green and white plastics items in favor of other colors could be useful for tortoise conservation. Comparable data on other species feeding on plastic, which are yet to be documented, would also be needed to make more comprehensive recommendations.

The different composition of debris found in tortoise feces relative to the environment may be explained due to the degradability of some materials (such as paper, that might be more difficult to find in feces) and the size and hardness of some others (rubber, material constructions, metals, and glass) that can be difficult for tortoises to ingest. Plastic particles were very frequently found in tortoise feces and may have been indiscriminately consumed by tortoises when taking mouthfuls of ground vegetation. We cannot reject that smell may also play a role in plastic ingestion (Boyer & Innis, 2019), as our team has directly observed tortoises trying to ingest silver containers and transparent plastic bags with human food leftovers. Thus, some ingestion of debris may possibly be explained by tortoises sometimes mistaking plastic debris for food. Regardless of the potential reason plastic is consumed, the digesta retention time observed in Galapagos tortoises of up to 28 days (Sadeghayobi et al., 2011) suggests the accumulation of these materials within the intestinal track of tortoises is cause for concern. Long digestions times could favor absorbance of chemical components such as EDCs that have the potential to lead to lesions and changes in the intestinal microbe composition (Deem & Holliday, 2022). These results are of major concern for tortoise health and long-term wellbeing and conservation of the species inhabiting human-modified areas.

Due to logistical constraints, we were limited in the area we could sample in the narrow road habitat and urban area that were also adjacent to thick vegetation. While we quantified the amount of debris in the environment, we did not quantify the amount of forage available between the park and anthropic habitats. The agricultural area for example has more pastural areas and resources for tortoises whereas roadside habitat has smaller patches of vegetation and higher ratios of debris dispersed throughout these patches, thus food availability may also contribute to the differences in some of our results with tortoises without enough food resources been more likely to eat artificial items.

A higher prevalence of tortoises with lesions on the carapace edges were found within the protected area. The characteristic of these lesions is suggestive of dog bite lesions and supports the negative impact that feral dogs have on tortoises within the National Park areas. In addition to dog attacks, during this study we observed tortoise entanglement and deadly car encounters; both indicating that tortoises face a number of anthropogenic threats that are not only limited to the human-modified areas.

Tortoise abundance was five times higher in the protected area when compared to the anthropic zone, highlighting the importance of habitat protection within the National Park areas. Within the human-modified areas, tortoises were more abundant in the agriculture, urban, and main road areas over the industrial and rural road areas. During their annual migration, at least 69% of the adult tortoises use the agriculture zone, remaining there for 150 days on average, at which time they return to the lowlands in the protected area (Pike et al., 2021). Tortoise density has been described to be strongly correlated to habitat structure and ground coverage, with preference towards abundant grass coverage with short vegetation and few shrubs (Pike et al., 2022a). In recent years, more tortoises have been observed foraging and resting near the northern limits of Puerto Ayora, and an increased number of car encounters have been reported, some of them with fatal consequences for tortoises. Recent evidence from GPS tracking tortoises shows that tortoises from Santa Cruz Island are more likely to use areas near roads and prefer pastures over native vegetation within the agricultural zone (Pike et al., 2022b). These behaviors align with our results and might explain why tortoises were also more abundant near roads and agricultural areas where fresh pastures are available. We hypothesize that these recently urbanized areas (such as El Mirador) offer several resources for tortoises all year around (i.e., water, grasses near the road, food waste) that together with habitat degradation and new roads and trail openings have enabled tortoise access from the protected areas to the edges of the town. This increased frequency of encounters between tortoises and humans and their debris may lead to significant consequences to tortoise health and challenges to their long-term survival.

Tortoise philopatry to farm and human-modified areas has been described by Pike et al. (2021, Pike et al., 2022a,b). In the current study, tortoise' recapture within anthropogenic areas was three times higher than in the National Park. The higher proportion of recaptures in the human-modified areas could be partially explained due to the higher sampling effort conducted (weekly vs. monthly). However, the recapture frequency indicated that tortoises remain in polluted environments for long periods of time and do not only use those areas in transit to protected and less polluted habitats. The most recaptures of tortoises in lowland areas corresponded to juveniles and subadults, which is also in agreement with the movement studies describing the direct correlation between migration and tortoise size (Blake et al., 2013). The proportion of males and females increased with elevation in both protected and human-modified areas, whereas the proportion of juveniles and subadults decreased with elevation. Both results are in agreement with migration patterns, having smaller and/or non-migratory individuals at lower elevations, especially during the dry season (Blake et al., 2013; Bastille-Rousseau et al., 2017). Taken together, this suggests that smaller tortoises that forage in the human-modified areas are also more likely to remain in these areas than migratory adults, and thus have a higher exposure to debris.

5. Conclusions

The present work describes and characterizes a relatively novel threat to the conservation of Galapagos tortoises. The finding of debris, especially plastics, in feces of free-living tortoises highlights the importance of taking actions that minimize garbage within the fragile environments of the Galapagos. As both the local population and number of tourists in the archipelago increases, the amount of debris generated and disposed of on the islands will increase, putting at risk the health and wellbeing of animals and humans. Plastic pollution is currently being studied and addressed by several institutions within the Galapagos marine environments (Mestanza et al., 2019; Zambrano-Monserrate & Ruano, 2020; Alfaro-Núñez et al., 2021; Jones et al., 2021; Jones et al., 2022), but few studies have been conducted on terrestrial ecosystems and the impacts of debris produced *in situ* with this the first study in the scientific literature assessing plastic ingestion. This study highlights some of the garbage hotspots near Puerto Ayora where management actions should be prioritized to protect foraging areas and tortoise health. More research is needed to understand to what extent plastic pollution is having on the health of tortoises and other wildlife in the Galapagos. We urge local stakeholders, including the community at large, to reinforce current policies and governance and to take immediate action to limit debris from entering the Galapagos environment. This should also include long-term education campaigns to promote behavioral changes within the local population and visitors to the iconic Galapagos archipelago.

CRediT authorship contribution statement

Karina Ramón-Gómez: Conceptualization, Methodology, Investigation, Writing – original draft, Visualization. **Santiago R. Ron:** Conceptualization, Methodology, Formal analysis, Writing – review & editing, Supervision. **Sharon L. Deem:** Conceptualization, Methodology, Writing – review & editing, Funding acquisition, Supervision. **Kyana N. Pike:** Formal analysis, Writing – review & editing, Visualization. **Colton Stevens:** Formal analysis, Writing – review & editing, Visualization. **Juan Carlos Izurieta:** Formal analysis, Writing – review & editing, Visualization. **Ainoa Nieto-Claudín:** Conceptualization, Methodology, Investigation, Formal analysis, Writing – original draft, Visualization, Project administration, Funding acquisition, Supervision.

Declaration of competing interest

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

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

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