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Plastic contamination of a Galapagos Island (Ecuador) and the relative risks to native marine species

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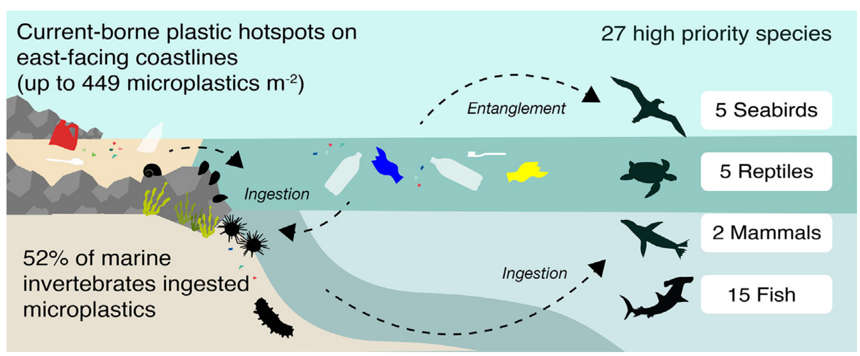
HIGHLIGHTS

- Plastic contamination was identified in all marine habitats surveyed in San Cristobal.
- Hotspots for beach plastics are on the eastern coast, up to 449 particles m^{-2}.
- Elevated microplastics in surface seawater around the harbour shows local inputs.
- Microplastics were found in 52% of marine invertebrates sampled (n = 123).
- 27 marine vertebrates scored at high risk of harm from entanglement and ingestion.

GRAPHICAL ABSTRACT



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ABSTRACT

Ecuador's Galapagos Islands and their unique biodiversity are a global conservation priority. We explored the presence, composition and environmental drivers of plastic contamination across the marine ecosystem at an island scale, investigated uptake in marine invertebrates and designed a systematic priority scoring analysis to identify the most vulnerable vertebrate species. Beach contamination varied by site (macroplastic 0–0.66 items·m^{-2}, microplastics 0–448.8 particles·m^{-2} or 0–74.6 particles·kg^{-1}), with high plastic accumulation on east-facing beaches that are influenced by the Humboldt Current. Local littering and waste management leakages accounted for just 2% of macroplastic. Microplastics (including anthropogenic cellulosics) were ubiquitous but in low concentrations in benthic sediments (6.7–86.7 particles·kg^{-1}) and surface seawater (0.04–0.89 particles·m^{-3}), with elevated concentrations in the harbour suggesting some local input. Microplastics were present in all seven marine invertebrate species examined, found in 52% of individuals (n = 123) confirming uptake of microplastics in the Galapagos marine food web. Priority scoring analysis combining species distribution information, IUCN Red List conservation status and literature evidence of harm from entanglement and ingestion of plastics in similar species identified 27 marine vertebrates in need of urgent, targeted monitoring and mitigation including pinnipeds, seabirds, turtles and sharks.

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1. Introduction

With the input of plastic contamination to aquatic systems predicted to triple in the next twenty years in a 'business as usual' scenario (Lau et al., 2020), negative impacts on marine food webs, ecosystem services, and coastal economies are of increasing global concern (Beaumont et al., 2019). Islands are host to 37% of all critically endangered species and have seen over half of recent extinctions due to the increased sensitivity of endemic species to anthropogenic stressors (Tershy et al., 2015). Although far from major population centres, some of the highest levels of plastic contamination have been reported on remote oceanic islands e.g. Henderson Island, where ca. 4500 plastic pieces$\cdot$m^{-2} were reported buried in surface beach sediment (Lavers and Bond, 2017). Plastics pose a potentially synergistic threat to fragile systems via physical habitat contamination, injury risk and as a potential vector for sorbed chemicals, pathogens and invasive species (Rochman et al., 2013; Bowley et al., 2021).

The Galapagos Islands, situated 930 km off the coast of Ecuador in the Pacific Ocean, are a UNESCO World Heritage Site famous for their endemic biodiversity. The interaction of several currents and strong upwelling systems drives the high productivity of the Galapagos Marine Reserve (Palacios, 2004), home to 22 marine species listed as endangered on the IUCN Red List. Anthropogenic pressures are mounting, with rising visitor numbers increasing the risk of invasive species introductions (Toral-Granda et al., 2017), escalating demand on resources including sanitation systems (Walsh and Mena, 2016) and the occurrence of illegal fishing, despite legislative protection (Carr et al., 2013). Isolated island fauna may be more vulnerable to marine pollution and ultimately extinction, due to reduced tolerance, reduced habitat availability, and therefore the inability to remove themselves from dispersed pollutants or chronic threats (Asaad et al., 2017). For example, mass marine iguana (*Amblyrhynchus cristatus*) mortality was reported following a major oil spill in 2001 despite exposure to only trace concentrations (Wikelski et al., 2002). Further, the Galapagos Marine Reserve is an important nursery ground for many species as well as being the second most important nesting and feeding habitat for the Pacific green turtle (*Chelonia mydas*) (Denkinger et al., 2013). Impacts from plastic contamination in Galapagos could have major ecological and socioeconomic consequences, particularly for the tourism industry that comprises 80% of the local economy (Pizzitutti et al., 2017).

Modelling approaches using virtual floating plastics transported on ocean surface currents have identified continental inputs as a major source of incoming plastic contamination to Galapagos, mostly from southern Ecuador and northern Peru where plastic leaked into the marine environment could arrive within a few months (Van Sebille et al., 2019). Together, Ecuador and Peru generated an estimated 304,000 t of mismanaged coastal plastic waste in 2010, projected to increase to 558,000 t by 2025 (Jambeck et al., 2015). Models suggest that only a small amount of plastic is entering Galapagos from known industrial fishing grounds but this does not reconcile with unpublished coastal clean-up data or archaeological analysis of macroplastic items that suggest maritime sources are likely a significant contributor (Van Sebille et al., 2019; Schofield et al., 2020).

Marine plastic contamination is a complex mixture of materials with a range of physical and chemical properties that can affect movement and accumulation in the environment and thus, potential impacts to ecosystems (Galloway et al., 2017). Microplastics, generally considered <5 mm, are of particular concern due to their high bioavailability, entering the marine environment from many sources such as river systems, agricultural run-off, wastewater or even via atmospheric deposition (Stanton et al., 2019). They may also be generated in the environment i.e. from fragmentation of larger plastic items, processes that are likely to be accelerated on Galapagos' beaches due to high equatorial solar irradiation levels, high oxygen availability and mechanical stress from wave action in the surf zone (Andrady, 2011; Chubarenko et al., 2020).

The aim of this study was to investigate the distribution, composition and environmental drivers of plastic contamination at an island scale to locate accumulation hotspots, and to develop a novel rapid assessment tool to identify at-risk marine vertebrates to facilitate conservation actions, particularly for those species found around accumulation hotspots. We focussed our sampling efforts on San Cristobal Island in the east of the Archipelago, situated in the pathway of the Humboldt Current (Fig. 1a). San Cristobal has areas of high conservation importance, including hosting the largest Galapagos sea lion (*Z. wollebaeki*) colony and two unique marine iguana (*A. cristatus*) subspecies (Miralles et al., 2017). Field sampling was conducted across both tourist and remote (no public access) sites (Fig. 1b) to investigate partitioning of plastic contamination across beaches, surface seawater, sediments and microplastic uptake in marine invertebrates across feeding modes. Linking field plastic contamination data to subsequent health impacts for individual organisms remains a significant challenge, given the multiple environmental stressors present in any habitat that might influence an individual's health meaning that it is not possible to assess any harm associated with the ingestion of microplastics simply from their presence within an individual at the point of sampling. It is also not feasible nor ethical to sample vertebrates, particularly those of endangered status. However, understanding the potential for harm from any plastic contamination present is essential for informing conservation and mitigation action, particularly in sensitive areas such as Galapagos. To address this issue, and to inform the prioritisation of research and mitigation efforts, we developed a systematic priority scoring analysis, based on species distribution information, IUCN Red list species vulnerability and harm data, to rank 710 Galapagos marine vertebrates threatened by exposure to plastic contamination to identify species at high risk.

2. Methods

2.1. Study site

San Cristobal (00°54′5.501 S, 89°36′47.537 W) is located in the east of the Galapagos Archipelago. The coastline is multi-use with a harbour town (Puerto Baquerizo Moreno; population approx. 8000 inhabitants, Fig. 1b, Site 7), popular tourist and fishing sites as well as remote areas that have no public access. The eastern coast is characterised by high energy rocky reef coastline interspersed with small sandy bays, primarily comprised of biogenic sediments e.g. urchin tests. Conversely, the western coastline is more sheltered and characterised by finer sandy beaches (see Supplementary Table 1 for site descriptions).

2.2. Field sampling

Seventeen sites were surveyed around the coast of San Cristobal including tourist sites and remote (no public access) areas with varying beach aspect (the direction in which a perpendicular line to the strandline travels e.g. SW aspect etc.). To gain a holistic understanding of plastic contamination, surveys were conducted for: (i) beach macroplastic (items and fragments > 5 mm), (ii) beach large microplastic (1–5 mm, sieved from the top 50 mm to sample particles per m^2), (iii) beach microplastics in whole sand (to sample all particles < 5 mm in 50 g from the surface 50 mm to include particles smaller than 1 mm missed by sieving), (iv) floating seawater surface microplastics (<5 mm) and (v) microplastics in benthic sediment (<5 mm). Environmental sampling took place in May 2018 working from a small local fishing boat doing daily excursions from Puerto Baquerizo Moreno.

2.3. Plastic surveys

2.3.1. Macroplastic

To control for variable beach morphology and patchy plastic accumulation, 2 × 50 m macroplastic transects were sampled to generate representative data for the whole beach. All visible plastic items and

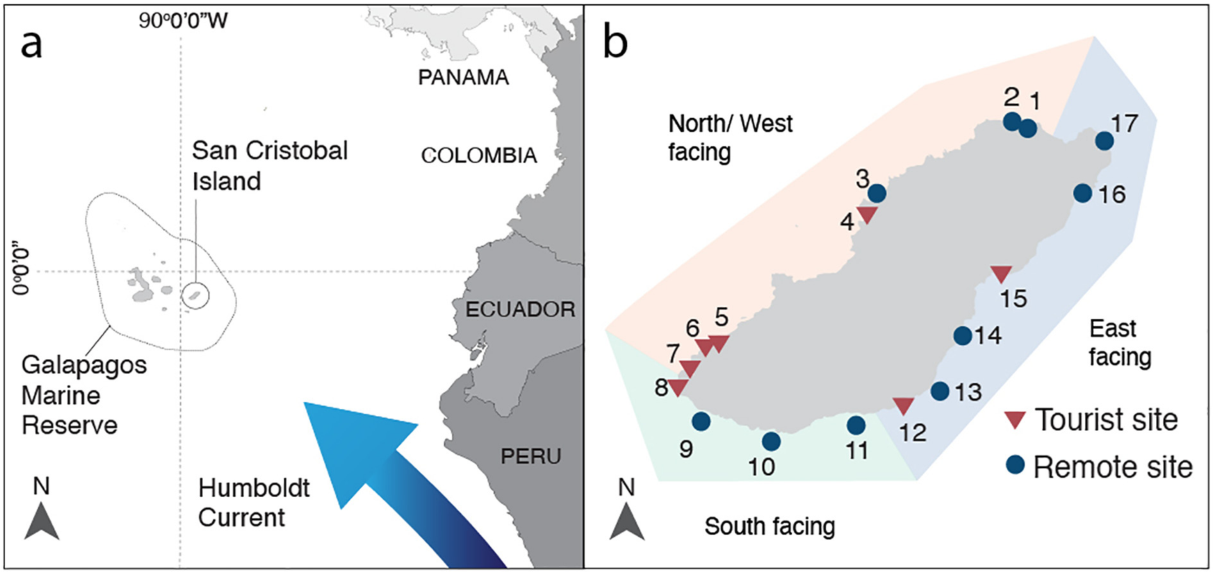

Fig. 1. Geographic location of study site: San Cristobal Island, Galapagos, Ecuador. (a) Geographical location of San Cristobal Island in the Eastern Pacific Ocean showing the Humboldt Current and the limits of the protected Galapagos Marine Reserve; (b) study sites coded by type (tourist sites/remote sites) and the aspect of beaches (i.e. north or west facing (grouped together due to sample size and similarity), south facing or east facing).

fragments (>5 mm) between the waterline and vegetation line were removed, counted and categorised according to possible source using a modified OSPAR protocol (OSPAR, 2010; Watts et al., 2017), see Supplementary Table 2 for categories. Beach area was calculated using satellite images (retrieved from Google Earth, January 2020) to convert data into items per square metre. A sub-sample of items from each location was taken for FTIR analysis to test for polymer similarity to smaller particle contamination (n = 137, approx. 5% of total sample).

2.3.2. Large microplastics (sieving)

Large microplastics (1–5 mm) were collected by sieving the top 50 mm of sand from five 50 cm × 50 cm quadrats at least 5 m apart on the strandline of each beach spread along the two macroplastic transects. Stacked 5 mm and 1 mm sieves were submerged in a bucket of seawater to ease sieving and cause most plastic particles to float. Suspected plastics were collected by hand or using forceps and stored in a 50 mL centrifuge tube. Seawater was checked for any floating particles before use. Whilst care was made to ensure that no visible microplastics were recorded, we acknowledge that there is a small chance of cross-contamination of microplastics from seawater as opposed to from the sand sampled. Centrifuge tubes containing the suspected plastics were washed out three times with deionised water and set out on filter papers for subsequent analysis. Particles <1 mm were discounted.

2.3.3. Sand sampling

To sample the smaller size fraction of beach plastic, triplicate 50 mL sand 'cores' were collected using centrifuge tubes from the surface 50 mm of beach sand at the strandline within the macroplastic transects. Sand samples were processed according to the density floatation protocol outlined by Coppock et al. (2017), with 50 g dry weight sediment suspended in a filtered zinc chloride ($ZnCl_2$) solution with a density of 1.5 g cm^{-3} that has a recorded recovery rate of 95.8% causing the majority of polymers to float in a custom-made Sediment-Microplastic Isolation unit. We acknowledge that this method will not recover plastics with a greater density than the media although these are unlikely to be numerous (Coppock et al., 2017). The surface compartment was poured off and filtered through 10 μm polycarbonate filters. Grain size was measured using a Saturn Digisizer using seperate 2 g samples previously sieved to 1 mm and digested for 24 h in 30% H_2O_2 solution at room temperature (approx. 23 °C).

2.4. Seawater surface sampling

Seawater surface tows of 2–10 min at 2 knots boat speed were undertaken in triplicate using an unweighted 200 μm plankton net with a cod end with a 200 μm mesh window and a flow meter, towing into the wind, away from the shoreline starting approx. 20 m offshore. GPS readings were taken at start and end of each tow. Samples were fixed in 4% formaldehyde solution in 500 mL Nalgene bottles. In sterile laboratory conditions, the formaldehyde solution was poured off through a 50 μm white nylon mesh leaving all solid matter in the sample bottle. The mesh was retained for inspection in a sealed petri dish. Approximately 100 mL of 20% filtered potassium hydroxide (KOH) solution was added to the remaining solid matter. Sample bottles were sealed and shaken vigorously before being heated at 40 °C for 48 h. Samples were vacuum filtered through a 50 μm nylon mesh and any remaining organic material was smeared on an extra mesh and sealed in petri dishes for later inspection.

The KOH solution used to digest the organismal soft tissue both in seawater samples (primarily plankton and fish eggs) and invertebrate samples, is acknowledged to damage certain polymers including polyesters such as polyethylene terephthalate (Cole et al., 2014). These polymers may therefore be underrepresented in our samples, or may in fact result in higher counts of smaller particles due to fragmentation during sample processing. Even non-digesting separation methods have been shown to impact upon particle identification (Jaafar et al., 2020) hence there are always trade-offs when choosing the approach for tissue microplastics analysis. A 20% KOH solution was selected here due to its relatively low impact compared to many other chemicals, often only causing deformation or discolouration rather than damage (Schirinzi et al., 2020) even at concentrations greater than those used here (Enders et al., 2017) in relation to its efficacy for digesting samples.

2.5. Benthic sediment sampling

Benthic sediment samples were collected in triplicate taking a 50 mL sample from a 250 cm^3 Van Veen Grab at 3–9 m depth at the finishing GPS position of the final seawater tow (approximately 20 m offshore). Benthic samples were processed following the same method as beach sand. Depths varied due to the difficulty of sampling sand patches at some sites.

2.6. Invertebrate sampling

Marine invertebrates were collected by hand in May 2018 and April 2019 during snorkelling, off beach rocks or on plastic litter found in the littoral zone. We selected seven representative species from six sites around San Cristobal comprising suspension and filter feeders including goose barnacles (*Lepas anatifera*) ($n = 7$), giant barnacles (*Megabalanus peninsularis*) ($n = 6$) and palmate oysters (*Saccostrea palmula*) ($n = 12$), grazers including rough-ribbed nerite snails (*Nerita scabricosta*) ($n = 23$), sculptured chiton (*Chiton sulcatus*) ($n = 4$) and Galapagos slate pencil urchins (*Eucidaris galapagensis*) ($n = 22$) and one species of deposit-feeding sea cucumber (*Holothuria kefersteini*) ($n = 49$). Species were selected according to abundance across sites ensuring that our extraction was unlikely to have ecological impact, with sampling numbers limited due to National Park restrictions. Invertebrates were thoroughly washed before freezing to minimise external contamination.

In the lab, invertebrates were defrosted, measured (maximum calliper) and dissected to remove soft tissues or just the digestive tract in the case of sea cucumbers, under clean conditions in a laminar flow fume hood and transferred to centrifuge tubes for oven-drying at 60 °C overnight, with open petri dishes with filters as atmospheric controls. Dry weight was recorded and samples were digested with 20% KOH solution at 40 °C for 48 h with shaking every 24 h. Samples were filtered through 10 μm polycarbonate filters (Whatman Nucleopore Hydrophilic Membrane).

2.7. Particle composition and FTIR analysis

Filters from environmental and organism samples were systematically examined using an Olympus MVX10 microscope and suspected synthetic particles were isolated, imaged, counted and categorised according to shape (fibre, fragment, foam, film, pellet) and colour. Fibres were checked to ensure visual identification criteria described by Hidalgo-Ruz et al. were met e.g. no visible cellular structures, consistent colours etc. (Hidalgo-Ruz et al., 2012). All particles were measured using Image J (length for fibres, feret diameter for all other particle types). All particles were analysed for polymer type using a PerkinElmer Frontier Fourier-transform infrared (FTIR) spectrometer using the attenuated total reflection (-ATR) universal diamond attachment for particles >1 mm or a PerkinElmer Spotlight 400 μFTIR Imaging System (MCT detector, KBr window) for particles < 1 mm. Particles were transferred onto a Sterlitech 5.0 μm silver membrane filter for analysis in reflectance mode (wavenumber resolution 4 cm^{-1}, 16 scans, range from 4000 to 650 cm^{-1}). Some fibres were isolated in a diamond anvil for analysis in transmission mode to improve spectra resolution. Linear normalisation and base-line correction tools from the Perkin-Elmers Spectrum™ 10 software (version 10.5.4.738) were used to further refine spectra. We used a general threshold of 70% library match for FTIR polymer analysis, from 8 different commercially available spectral libraries covering polymers, polymer additives and adhesives by PerkinElmer (adhes.dlb, Atrpolym.dlb, ATRSPE~1.DLB, fibres.dlb, IntPoly.spl, poly1.dlb, polyadd1.dlb and POLYMER.DLB). The top ten closest matches were analysed visually to improve our confidence in results. Due to their artificial composition and current poor understanding of their biological impacts, we have included anthropogenically modified cellulosic polymers (e.g. rayon, viscose) in our counts as per Hartmann et al. (2019).

2.8. QA/QC

In the field, the plankton net was deployed suspended on a beam around 3 m off the side to minimise boat-based contamination blowing into the net. All kit was thoroughly cleaned between replicates. Procedural blanks were undertaken in the field during seawater surface sampling by suspending the net above the water surface for the tow duration (10 min) after cleaning, and then washing out the net into a sample bottle to capture any potential contamination retained in the net or any atmospheric contamination. Further, a damp filter paper, placed in a petri dish was held at the height of the net opening, forward of the net to also control for airborne contamination underway.

All chemicals were filtered prior to use and all field equipment was rinsed in filtered DI water before deployment. Procedural blanks were undertaken for all processed sample types including seawater samples, sediment samples, beach sand samples and invertebrates. The same chemicals and plastic laboratory consumables were used to undertake these blank runs to control for potential contamination. Nitrile gloves and cotton clothing were worn in the field and laboratory. In the laboratory, all surfaces and equipment were thoroughly cleaned down with ethanol (three times) or rinsed with Milli-Q (three times) before each processing step. Sterile plastic equipment was used directly from packaging and metal and glass materials were used in favour of plastics where possible and feasible. All samples and equipment were covered whenever possible by aluminium foil. Potential airborne contamination was controlled for by leaving exposed in the lab during any sampling and procedural steps.

Each of the procedural and atmospheric blanks underwent the same processing steps as environmental samples. Contamination was low but measurable, in seawater samples, 3 out of 12 atmospheric blanks had one black cellulosic fibre and 1 out of 12 had two fibres, one black, one blue cellulosic. In beach and benthic sediment samples, 1 out of 8 atmospheric blanks had a black polyester fibre and 7 out of 14 procedural blanks had 1 blue polyacrylamide or cellulosic fibre recovered. No contamination was recorded during processing of invertebrate samples (blanks = 8). To control for this potential contamination, the mean number for each particle category across all the relevant blanks was subtracted from all data prior to further analysis and is not included in any data presented.

2.9. Priority scoring

As vertebrates could not be sampled directly, species lists for marine vertebrates of the Galapagos Islands with information on distribution and origin were retrieved from the Charles Darwin Research Station Natural History Collections database collated from sightings over several decades (https://www.darwinfoundation.org/en/datazone), incorporating 710 species. Species were given a distribution score (S^D): invasive ($S^D = 0$), unknown ($S^D = 1$), migratory or native ($S^D = 2$) or endemic ($S^D = 3$). The IUCN Red List status of each species was retrieved from the IUCN database (https://www.iucnredlist.org/) to generate a conservation score (S^C): data deficient, not evaluated, least concern ($S^C = 1$), near threatened, vulnerable ($S^C = 2$) or endangered, critically endangered ($S^C = 3$). To establish literature evidence of harm from entanglement (S^{EL}) or ingestion (S^{IL}) we undertook a literature search for each species using Web of Science, searching by genus and the term "plastic" including grey literature e.g. for necropsy data resulting in 138 studies that showed likely harm. The literature evidence was separated into entanglement and ingestion related publications, then organised into three categories describing the amount of evidence available surrounding the species interaction with plastic, considering the volume of published literature and the study design and scope. The categories are as follows: No Evidence: *There is no current evidence on the effects of marine plastics that can be correlated to the given species* (S^{EL} or $S^{IL} = 1$); Moderate: *There is evidence that demonstrates the species, or a species of the same genus, has had interactions with marine plastics which may have resulted in non-lethal effects, or affected survival* (S^{EL} or $S^{IL} = 2$); Major: *There are multiple sources of evidence that demonstrate the species has had major interactions with marine plastics which have resulted in severe injury or death* (S^{EL} or $S^{IL} = 3$). Where comparisons were made using published information on species in the same genus, a maximum score of S^{EL} or $S^{IL} = 2$ was awarded as they are likely to interact with marine plastics in a similar way as the closely related species, yet there is currently no evidence on the species and thus a score of "3" (major impacts) is unjustifiable. The only exception to this rule was the marine iguana (*Amblyrhynchus cristatus*) as they are the only marine iguana species on earth. Therefore, the evidence gathered on turtle species was used as a comparison as they are also herbivorous reptiles native to the region, and thus are likely to have experienced similar interactions with marine plastics. To calculate the priority species at high threat from marine plastics entanglement (1) and ingestion (2) we used the following simple equations:

$$E = S^D \times S^C \times S^{EL} \quad (1)$$

$$I = S^D \times S^C \times S^{IL} \quad (2)$$

2.10. Statistical analyses

All statistical analysis was undertaken in RStudio Version 1.1.463 (R Core Team, 2014). Differences in abundance were statistically compared using a Kruskall-Wallis test with a post hoc Dunn's test to determine significant differences. Spearman's Rank Coefficient was used to test for correlation between abundances between habitats. We used generalised linear modelling (GLM) to model which of our factors; beach aspect (north/west, south, east), windward vs leeward orientation, site usage (tourism, remote), distance from port and grain size had an impact on the accumulation of microplastics based on counts of (i) beach macroplastic, (ii) sieved beach microplastics, (iii) synthetic particles in whole sand samples, (iv) synthetic particles at the seawater surface and (v) synthetic particles in benthic sediments. A negative binomial GLM with a log link function was selected due to over-dispersed data and a separate model conducted for each response variable. Optimisation was achieved by backwards step-wise deletion of the least significant variable (determined by the highest *p* value) until the lowest Akaike Information Criterion (AIC) value was achieved and the fewest explanatory variables were identified. No interaction terms were included as they had a negligible effect on models. To validate the models, a dispersion test was undertaken to verify correction of overdispersed data and residuals were plotted to ensure an acceptable level of normality, homoscedasticity, and there were no excessively influential observations (Supplementary Fig. 1). Top ranked models were defined as models ΔAIC ≤ 2 units of the best supported model. Models that could not be fitted to a linear model were omitted.

3. Results and discussion

3.1. Macroplastic on the beach

Macroplastic contamination (items and fragments >5 mm) was recorded on 13 out of 14 sandy beaches sampled, with a total of 4610 items collected from the back-beach vegetation line to the water line along 100 m transects. Abundance was more than five-fold higher on east-facing beaches exposed to the Humboldt Current (mean 0.27 ± 0.12 items·m^{-2}) than on southern (0.05 ± 0.04 items·m^{-2}) or northern and western-facing beaches (0.02 ± 0.01 items·m^{-2}, Fig. 2). A GLM examining possible environmental drivers of plastic abundance by site using explanatory variables such as beach aspect, site usage, grain size and distance from harbour, revealed that none of the measured parameters were statistically significant drivers of macroplastic distribution (Table 1). Assigning source (i.e. usage and responsible industry) and the mechanisms of release and pathways within the environment are difficult for plastics, for example, a bottle could be littered on the beach, thrown overboard or carried on currents from riverine inputs. Only items that did not show evidence of prolonged marine exposure e.g. no epibionts, no yellowing, no degradation of labels etc. similarly to Thiel et al. (2013), were assigned to 'local' littering and waste management leakages, which represented just 2% of the items recorded. Tourist beaches were generally clean, as described by Mestanza et al. (2019), a likely result of small population size, elevated environmental expectations of visitors and good provision of bins and awareness messaging although due to accessibility, tourist beaches also tend to be on sheltered coasts that are less likely to receive incoming current-borne contamination.

The majority of beach macroplastic was classified as 'unsourced' (88%) assumed to be primarily from external sources to the Galapagos Marine Reserve; comprising mostly weathered hard plastic fragments (49% of total macroplastic, n = 2240) (Fig. 2). Drinks bottles, caps and sealing rings were also common (53% of unsourced items, n = 1248). Maritime items accounted for 10% of macroplastic by frequency (n =

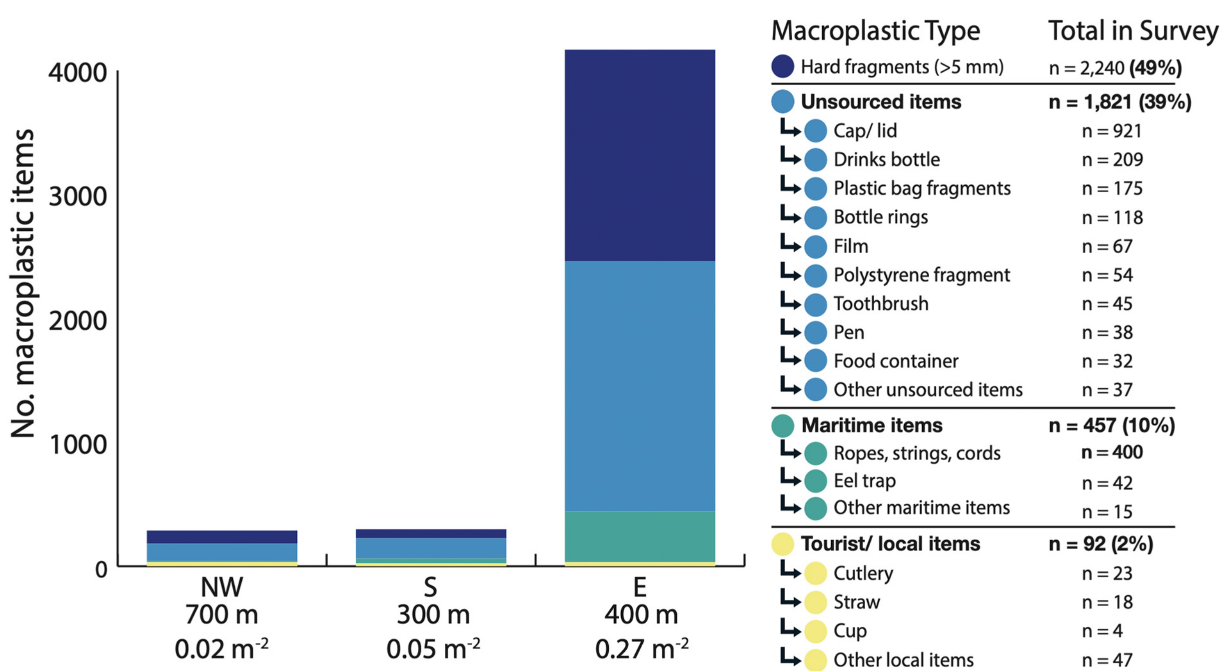

Fig. 2. Composition of beach macroplastic found on San Cristobal Island, Galapagos, Ecuador. Items recovered from the beach surface across 14 north/west, south and east facing beaches (NW, S, E) with total distance surveyed (m) and mean litter density (items·m^{-2}) labelled for each group. Totals and percentage of each item source type are reported across the full 1.4 km surveyed coastline in the key along with a breakdown of major contributing items.

Table 1
Summary results of best-fit negative binomial generalised linear models (GLMs) for environmental data. Explanatory variables explored included beach aspect (north/west, south, east), distance from port, windward vs leeward orientation and grain size. Statistically significant explanatory variables are denoted with *. AIC = Akaike's Information Criterion used in the step-wise ranking of models and OD = overdispersion calculated for the model.

Response variable	Explanatory variables	Estimate	Standard error	Z value	p value	AIC	OD
Macroplastic items	Intercept	−4.187	1.372	3.053	0.0027*	43.345	0.36
	Beach aspect (South)	1.354	1.423	0.780	0.43537		
	Beach aspect (East)	2.651	1.438	1.844	0.06518		
Sieved microplastic (particles 1–5 mm)	Intercept	0.15157	0.512	0.296	0.7671	467.41	0.98
	Usage 2 (Remote)	−0.71625	0.586	1.221	0.222		
	Distance from port	0.02986	0.030	1.700	0.891		
	Beach aspect (South)	1.58152	0.704	2.246	0.0247*		
	Beach aspect (East)	3.61741	0.652	5.544	<0.001*		
Seawater (particles < 5 mm)	Intercept	−1.019	0.485	−2.102	0.036*	40.92	0.62
	Distance from port	−0.029	0.019	−1.533	0.125		

457, mostly ropes), found along all coastlines. Given the protection within the marine reserve from industrial fishing and the small size of the artisanal fleet, gear loss and irresponsible disposal appears to be low locally. There is evidence of some connectivity with continental fisheries, as floating polypropylene eel traps, a gear not used in Galapagos, were recovered from one east-facing beach (n = 20; Site 16, Fig. 1b). A beached Fish Aggregating Device (FAD) was also observed; although illegal in Galapagos, FADs have been increasingly reported in recent decades (Boerder et al., 2017) and represent a ghost-fishing risk whilst in the water, an entanglement risk on the beach and a major future source of microplastics. By way of polymer, more than 92% of macroplastic sampled (5% sub-sample analysed by Fourier Transform Infra-red spectroscopy; n = 137) was categorised as being derived from petrochemical-based polymers (Fig. 3bi).

3.2. Microplastic on the beach

Large microplastics (1–5 mm) sieved from the surface 50 mm were found at 11 out of 15 sites and >95% were from secondary sources i.e. a result of environmental fragmentation (n = 1694; 78% fragments, 13% fibres, 4% films and 2% pellets). The mean concentration was 53 ± 30 particles·m^{-2}, but distribution was patchy (Fig. 3aii). A GLM identified beach aspect as a significant driver of beach microplastic accumulation (p < 0.001, Table 1) with abundance significantly higher on east-facing beaches. The highest contamination was 808 particles·m^{-2} collected from one part of Punta Pitt beach (Site 17, Fig. 1b). This high concentration is similar to those recorded in Easter Island situated in the plastic accumulation zone of the South Pacific Gyre (805 particles·m^{-2} in the top 2 cm) (Hidalgo-Ruz and Thiel, 2013) and for the Azorean Archipelago on the edge of the North Atlantic Gyre (averaging >500 particles·m^{-2} in the top 10 mm) (Pham et al., 2020). Eighty percent of large microplastic was made up of floating petrochemical-based polymers polyethylene and polypropylene (Fig. 3bii) and were generally white/ black/ blue fragments or blue/green fibres (see Supplementary Fig. 2). This similarity in composition and correlation in abundance of macroplastic and large microplastic (Spearman's rank correlation coefficient; R_s = 0.794, p < 0.001, df = 14) aligns with the hypothesis that macroplastic may well be fragmenting in situ as has been described in other island systems (Ryan and Schofield, 2020). Fragmentation is quicker in the beach environment than in seawater and could be accelerated by strong equatorial UV in the Galapagos Islands (Andrady, 2011).

The average concentration of microplastics in whole sand samples was 74.6 particles·kg^{-1} and there were no sites with significantly higher abundance (Fig. 3aiii). There was no correlation between the concentration in whole sand samples and the concentration of sieved microplastics or macroplastics. Fibres were much more commonly reported in whole sand samples than were found during sieving (40% of the 173 extracted particles). Fragments made up 53% and were a similar polymer composition to sieved samples and macroplastics, i.e. mostly polyethylene and polypropylene suggesting a possible shared source (Fig. 3biii). Fibres were mostly anthropogenic cellulosics (60%), generally associated with textiles (PlasticsEurope, 2018). Whilst there was a lack of significant differences between sites, the same trend is evident of higher numbers in the east-facing beaches.

Our findings highlight the importance of location in informing the likelihood of microplastic deposition on beaches and therefore risk to wildlife. Punta Pitt is east-facing and therefore directly open to the Humboldt Current. Punta Pitt is also one of the few sandy beaches with sand to the waterline (some are raised back beaches) also promoting its position as a depositional environment. Whilst our grain size analysis did not show any correlation between microplastic size and grain size (as shown in other studies also e.g. Urban-Malinga et al., 2020), sediment dynamics will likely play a role in partitioning of plastics as shown in riverine and estuarine sediments (Waldschläger and Schüttrumpf, 2020). The marine environment is not dominated by one major process (fluvial flow direction) such as in rivers. Physical processes such as wave action and tidal patterns are considered key to the accretion of microplastics on beaches, and further, aspect, slope, and shape of beach will likely play important roles in accumulation (Mathalon and Hill, 2014). This complex relationship is demonstrated by the fact that sites a few kilometres away from Punta Pitt (Sites 1–4, Fig. 1b) had no large microplastic recorded at all (Fig. 3aii). As these are west facing beaches they likely do not receive the inputs Punta Pitt does.

3.3. Seawater surface microplastic

Microplastic contamination of the seawater surface was measurable at low concentrations at all 17 sites with an island average of 0.16 ± 0.03 particles·m^{-3} (Fig. 3aiv–v). No significant explanatory variables were identified by GLM when models included beach aspect, windward vs leeward orientation, site usage or distance from harbour (Table 1). The harbour (Site 7, Fig. 1b) had significantly higher seawater surface contamination with a concentration of 0.89 particles·m^{-3} (Kruskall-Wallis test; H = 33.59, df = 16, p = 0.006) (Fig. 3aiv) suggesting local inputs such as wastewater outfalls, boat activity, and surface runoff from the largest population centre on the island may be driving this increase in seawater surface microplastic at this site. Seawater surface particles (n = 373) included polypropylene and polyethylene fragments (32%), synthetic cellulosic fibres (24%), polyester fibres (11%), polypropylene fibres (11%) and nylon fibres (7%) suggesting a mixture of sources (Fig. 3biv).

Overall, our floating microplastic numbers are low compared to studies across the globe. In a study of the Macaronesian islands in the North Atlantic, floating plastic numbers ranged from 21 to 894 particles·m^{-3} (Herrera et al., 2020). The low numbers of floating microplastics may be due to the great distance they would have to travel to reach the Galapagos Islands, if originating from continental South America, with many being lost en-route due to their inherent density and for floating polymers, biofouling along the way (Fazey and Ryan, 2016). Furthermore, the prevailing equatorial current is likely to carry

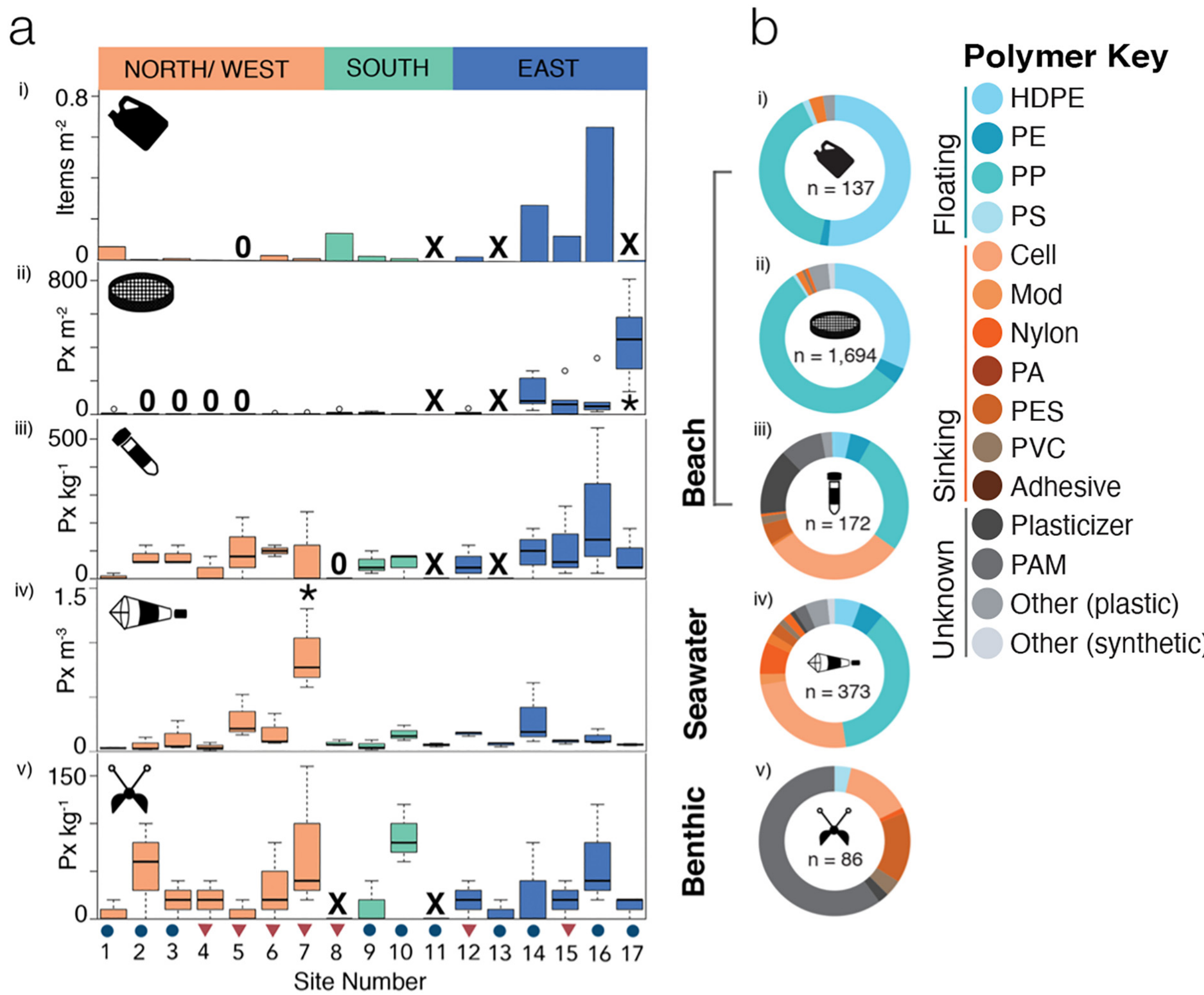

Fig. 3. Abundance and polymer composition of macroplastic items and synthetic particles (including microplastics) around San Cristobal Island, Galapagos, Ecuador. (a) Abundance of (i) beach surface macroplastic (items·m^{-2}); (ii) sieved large microplastics (1–5 mm) particles (hereby denoted by px) m^{-2}); (iii) beach sand (px·kg^{-1}); (iv) seawater surface (px·m^{-3}); (v) benthic sediment (px·kg^{-1}). Significant values (Kruskall-Wallis Test with Dunn's Posthoc) indicated by asterisks. 'X' indicates sites where sampling was not possible and zero values (0) are labelled. Sites are grouped by beach aspect (north/west, south or east) and labelled as remote sites (blue circles) and tourist sites (red triangles) (see Fig. 1b for location). (b) Polymer composition of macroplastic items and synthetic particles (denoted by n) recovered from environmental samples as verified by Fourier Transform Infrared Spectroscopy (FTIR) for beach (i–iii), seawater surface (iv) and benthic sediment (v). Polymers are labelled as floating (blue shades), sinking (orange shades) or unknown (grey). Polymer key: HDPE = high density polyethylene, PE = polyethylene (various), PP = polypropylene, PS = polystyrene, Cell = Cellulosic (synthetic), Mod = modacrylic, PA = polyamide, PES = polyester, PVC = polyvinyl chloride, PAM = polyacrylamide.

floating microplastic away from Galapagos and towards the subtropical gyres as described by Van Sebille et al., who also highlight that almost no particles in their model arrived in Galapagos from the North or South Pacific subtropical gyre accumulation zones (Van Sebille et al., 2019). This is in direct contrast to the Azores where it is likely that most microplastic inputs come from the gyres, particularly during storm surges displacing the gyre microplastics and pushing them onto the beach at Porto Pim (in extremely high concentrations of up to 9338 ± 386 items·m^{-2}) (Pham et al., 2020).

3.4. Benthic sediment

Benthic sediment contamination was not significantly higher around the populated harbour. The island mean was 35.8 ± 6.8 particles·kg^{-1}, (range 6.7–86.7 particles·kg^{-1}, Fig. 3av), less than half the concentration recovered from beach sediment. This level of contamination is low compared to other studies. Jahan et al. (2019) recorded plastic contamination of benthic sediments off the coast of New South Wales in Australia, taken at similar depths to our study ranging between 83 and 350 particles·kg^{-1}. There are reports of much greater deep sea microplastic concentrations such as 13,600 particles·kg^{-1} in the Great Australian Bight at >1600 m depth however these hotspots are dictated by large scale deep sea physiographic processes and perhaps mirror the differences found in the way micro- and macroplastic seem to behave on the surface (Barrett et al., 2020). Our benthic sediments, taken in relatively shallow waters will be much more likely resuspended under storm conditions perhaps creating microplastic stores on beaches rather than in shallow water sediments.

As regularly reported in other studies (e.g. Scott et al., 2019), over 90% of benthic microplastic contamination was fibres. The closest spectrum match for 58% of fibres was polyacrylamide although these are suspected to be more likely cellulosic polymers as polyacrylamide is generally a gel and not commonly found in the environment (Xiong et al., 2018). Suspected anthropogenic cellulosics (14%) and polyester fibres (14%) were reported (see Fig. 3bv), both high density polymers that are more likely to sink. Although floating plastics of all sizes might enter the marine reserve, denser polymers may be more likely to sink out of the water column in coastal sediments, being incorporated in sediment transport processes closer to continental sources (Zhang, 2017). If this is true, benthic contamination in Galapagos is more likely to be locally generated and warrants further investigation of wastewater, agricultural run-off and contamination of terrestrial systems.

3.5. Contamination of Galapagos marine invertebrates

All seven marine invertebrate species sampled contained synthetic particles and all but the chiton (*Chiton sulcatus*) contained petrochemical-based microplastics. Overall, mean incidence of ingestion was 52% across all individuals (n = 123). Giant barnacles (*Megabalanus peninsularis*) had the highest proportion of individuals containing microplastics (83%) followed by pencil urchins (*Eucidaris galapagensi*) (60%) (Fig. 4). There were no significant drivers influencing particle uptake in marine invertebrates when tested by GLM and no correlation was found between number of particles and invertebrate dry weight (Supplementary Fig. 3, Supplementary Table 4) acknowledging that our data is limited to small sample sizes for some species. Particle characteristics including shape, colour and size varied between feeding groups as discussed below (summarised in Supplementary Fig. 2 and Fig. 4). Of the 177 suspected synthetic particles extracted from invertebrates, 50% (89 particles) were disregarded after FTIR analysis, and therefore not included in these data, due to identification as natural polymers or weak library spectral matches (defined as <70% match to library polymers). This rejection rate is much higher than for particles extracted from environmental media (<10%). This suggests that particle identification and isolation is more challenging in organisms, possibly due to transformations that organismal gut fluids and feeding mechanisms may have exerted on the particles, the additional methodological digestion steps, generally smaller particles or confusion with biological structures.

Suspension and filter feeders are exposed to particles in suspension, sinking through the water column or those resuspended from the seabed. All of the microplastic particles found within the filter feeding species sampled here, comprising goose barnacles (*Lepas anatifera*), giant barnacles (*M. peninsularis*) and palmate oysters (*Saccostrea palmula*), were fibres (19 particles extracted), with mean abundance per individual of 0.71 ± 0.29, 1.17 ± 0.31 and 0.67 ± 0.30 respectively (Fig. 5a). This is a relatively low level of contamination compared to other studies, particularly for oysters where up to 35 particles per individual have been recorded (Wu et al., 2020). The length of fibres ranged from 367 to 2508 μm in goose barnacles, 519 to 8348 μm in giant barnacles, and from 733 to 12,572 μm in oysters. Extracted fibres were mostly higher density polymers such as anthropogenic cellulosics (70%) and nylon (11%) (Fig. 5b), similar to the polymer composition of particles from benthic sediment, echoing the relationship observed in a UK study of mussels (*Mytilus edulis*) (Scott et al., 2019). Fibres have a larger surface area than fragments and a greater propensity to become bio-fouled and sink which may increase bioavailability to filter feeders that also play a role in modulating microplastic pathways by drawing down particles to the benthos (Schwarz et al., 2019). Fibres may also be more likely to be retained in organisms or entangled in morphological structures (Welden and Cowie, 2016). This was observed in three goose barnacles in our study, where >1.5 mm clumps of green polypropylene fibres were extracted (see Fig. 4) suggesting potential physiological impacts from either gut or gill obstruction, due to the amount accumulated relative to the size of the animal (mean carapace length 12 mm).

Particles within grazers (n = 34) were more diverse in terms of shape (53% fragments, 44% fibres), colour and polymer type (Fig. 5b, Supplementary Fig. 2) compared to those found within filter feeders. Cellulosics were again the most common polymer (26%), but polyester (13%), polypropylene (13%), polyethylene (10%) and adhesives (19%) were also found. This suggests that grazers may have more potential microplastic uptake routes, directly from the environment or indirectly via the consumption of particles associated with dietary items such as algae (Gutow et al., 2016) or from grazing on biofilms formed on macroplastic (Porter et al., 2019). Three gastropod snails collected from beach macroplastic contained polypropylene fragments with scouring marks possibly caused by radula. Suspected bite marks were also observed on polypropylene fragments recovered from urchins (n = 4, see Fig. 4) very similar to those seen in Porter et al. (2019). This represents an ingestion pathway and also a process of mechanical fragmentation, as demonstrated in laboratory studies where a single urchin grazing on macroplastic produced >90 fragments in 10 days (Porter et al., 2019). The gastropod snails (*Nerita scabricosta*), chitons (*C. sulcatus*), and pencil urchins (*E. galapagensis*) all had an average

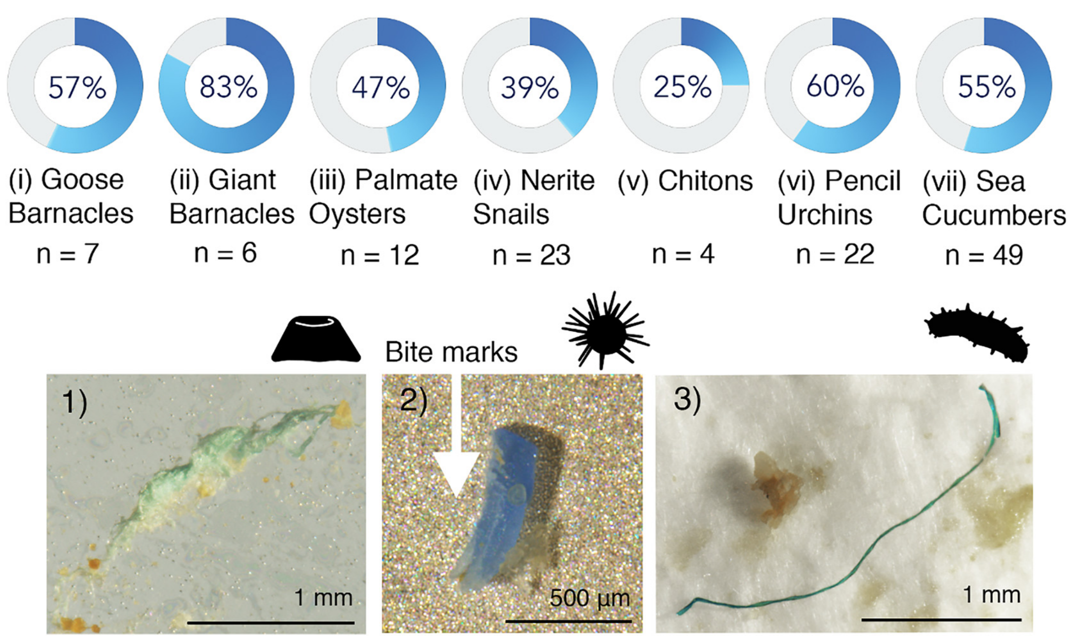

Fig. 4. Synthetic particle uptake in marine invertebrates. Percentage of organisms containing synthetic particles (including petrochemical microplastics and anthropogenic cellulosics) and number of individuals sampled (n) across seven species: (i) goose barnacles (*Lepas anatifera*), (ii) giant barnacles (*Megabalanus peninsularis*), (iii) palmate oysters (*Saccostrea palmula*), (iv) rough-ribbed nerite snails (*Nerita scabricosta*), (v) sculptured chiton (*Chiton sulcatus*), (vi) slate pencil urchins (*Eucidaris galapagensis*), (vii) sea cucumber (*Holothuria kefersteini*); images of typical particles recovered: (1) a clump of green polypropylene fibres recovered from a goose barnacle, (2) a blue polypropylene fragment with suspected bite marks recovered from a slate pencil urchin and (3) a blue/green synthetic cellulosic fibre recovered from a sea cucumber.

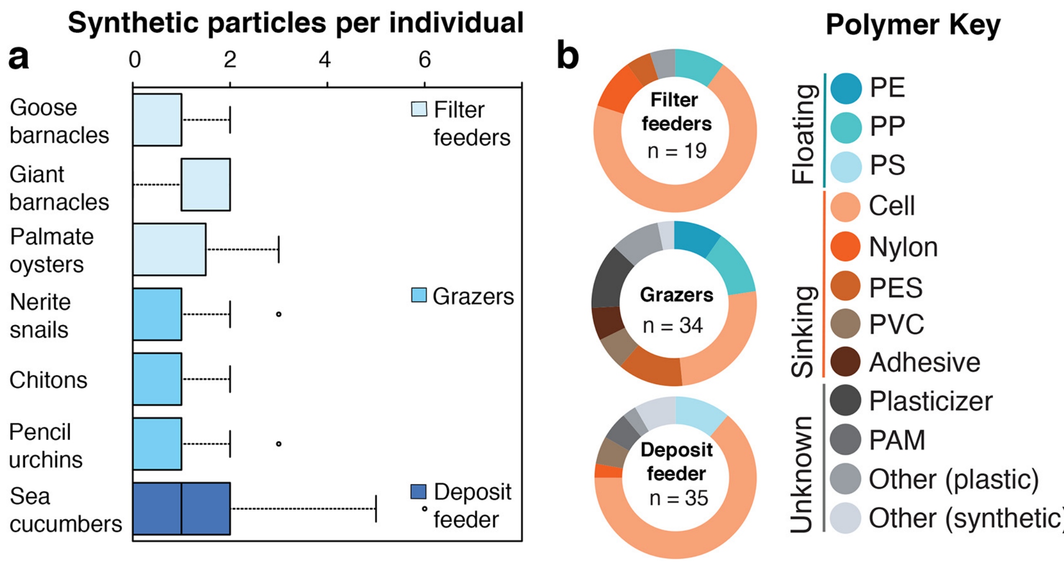

Fig. 5. Synthetic particle abundance and composition in marine invertebrates. (a) Mean synthetic particles per individual averaged over all sampled species grouped by feeding mode; (b) polymer composition of particles (denoted by n) extracted from filter feeders, grazers and surface deposit feeders. Polymer key: PE = polyethylene (various), PP = polypropylene, PS = polystyrene, Cell = cellulosic (synthetic), PES = polyester, PVC = polyvinyl chloride, PAM = polyacrylamide.

number of particles per individual less than one (0.65 ± 0.19, 0.5 ± 0.5, and 0.68 ± 0.18 respectively). Ingested plastics ranged in size from 83 to 2016 μm within the gastropod snails, for chitons 131–1174 μm, and for urchins 106–3270 μm. These data are in a similar range to those measured in benthic invertebrates including gastropods and asteroids in the Arctic where species means varied from 0.04 to 1.67 particles·ind^{-1} (Fang et al., 2018). Microplastic contamination data in the literature is scarce for urchins, however Bour et al. (2018) found 0.45 microplastic particles·ind^{-1} in spiny mudlark urchins (*Brissopsis lyrifera*) in a Norwegian fjord and Feng et al. (2020) found high levels of contamination of three species of urchin along the coastal areas of northern China (mean 4.94 particles·ind^{-1}).

The sea cucumber (*Holothuria kefersteini*) specimens analysed contained a mean of 0.99 ± 0.34 particles·ind^{-1}, with higher contamination observed in specimens from the polluted, east-facing beach of Rosa Blanca (mean 2.54 ± 0.61 particles·ind^{-1}, 100% individuals with ingested plastics, n = 11, see Supplementary Table 3). These findings are similar to holothurians elsewhere although by no means the highest; Renzi et al. (2020) report particle concentrations of 3.8–6.0 particles·ind^{-1} in the Aeolian Archipelago in the Mediterranean. Extracted particles were a mix of fibres (69%) and fragments (31%) and the most common polymers were synthetic cellulosics (64%). Sea cucumbers were the only invertebrate to have ingested polystyrene (11%), a rare polymer in our study. This differs from the composition of the sediments they inhabit, perhaps suggesting selectivity in their uptake of microplastics, as shown in laboratory studies of deposit feeding species (*Holothuria* spp.) (Graham and Thompson, 2009). The feeding mode of sea cucumbers makes them potentially good indicators for benthic microplastic contamination due to their high throughput of ingested sand and available evidence to date suggests they may well bioaccumulate plastics above ambient levels (Renzi and Blašković, 2020).

A number of studies have suggested that anthropogenic fibres might exert more toxicological harm, due to their aspect ratios allowing a greater contact surface area with tissues, than fragments. This was demonstrated by Gray and Weinstein (2017) in the daggerblade grass shrimp (*Palaemonetes pugio*) whereby polypropylene fibres exerted significant mortality across size ranges whereas fragments or spheres only exerted mortality in a size dependant manner. The microplastics found in invertebrates in our study were at the top end of what are usually used in microplastic toxicological exposures. Burns and Boxall (2018) report that 95% of all studies (n = 91) used particles <100 μm) and yet those found in our invertebrates had an average length across all species of 1329 ± 179 μm. It seems pertinent therefore, given the size dependent effects found in the literature (broadly, larger size plastics exert more of a toxicological effect), that laboratory exposures utilising larger particles are undertaken to try to elucidate potential harm from particles more reflective of what we find in wild marine invertebrates and their habitats.

All raw data for beach, water, sediment and invertebrate contamination has been made available at https://doi.org/10.5061/dryad.dbrv15f1f.

3.6. Rapid assessment of plastic contamination impacts for Galapagos marine vertebrates

A total of 138 published studies were included in the assessment of literature evidence of harm to marine vertebrates from plastic contamination. Our inclusion criteria were limited to studies documenting entanglement or ingestion encounters that clearly show harm, i.e. injury or death and thus studies only showing uptake were not included. Low scores do not necessarily equate to low risk in this analysis, rather that potential negative impacts are unknown due to a lack of evidence. Endangered and endemic species are most likely to score highest (see Supplementary Table 5 for scoring criteria and Supplementary Table 6 for examples illustrating the scoring mechanism). Twenty-seven species had a score greater than 10 (maximum score 27) indicating likelihood of severe injury or death from plastic ingestion or entanglement upon encounter. These included 15 fish species (13 shark spp.), five reptiles (marine iguana and four sea turtle spp.), five seabirds and two mammals (both pinnipeds) (Fig. 6, listed in Supplementary Table 7).

This tool represents a useful starting point for prioritising risk assessments for species at an Archipelago scale but does not represent a risk assessment itself due to the lack of data to accurately predict exposure. The Galapagos Marine Reserve is made up of several biogeographic zones that are differentially influenced by oceanographic currents and upwelling, that in turn impact species distributions and marine communities (Edgar et al., 2004a; Tompkins and Wolff, 2016). We acknowledge that this tool could be improved in the future with the incorporation of environmental data across biogeographic zones so that the types and density of plastics found can be mapped against the ranges of species to establish risks. In the following section, we discuss the species

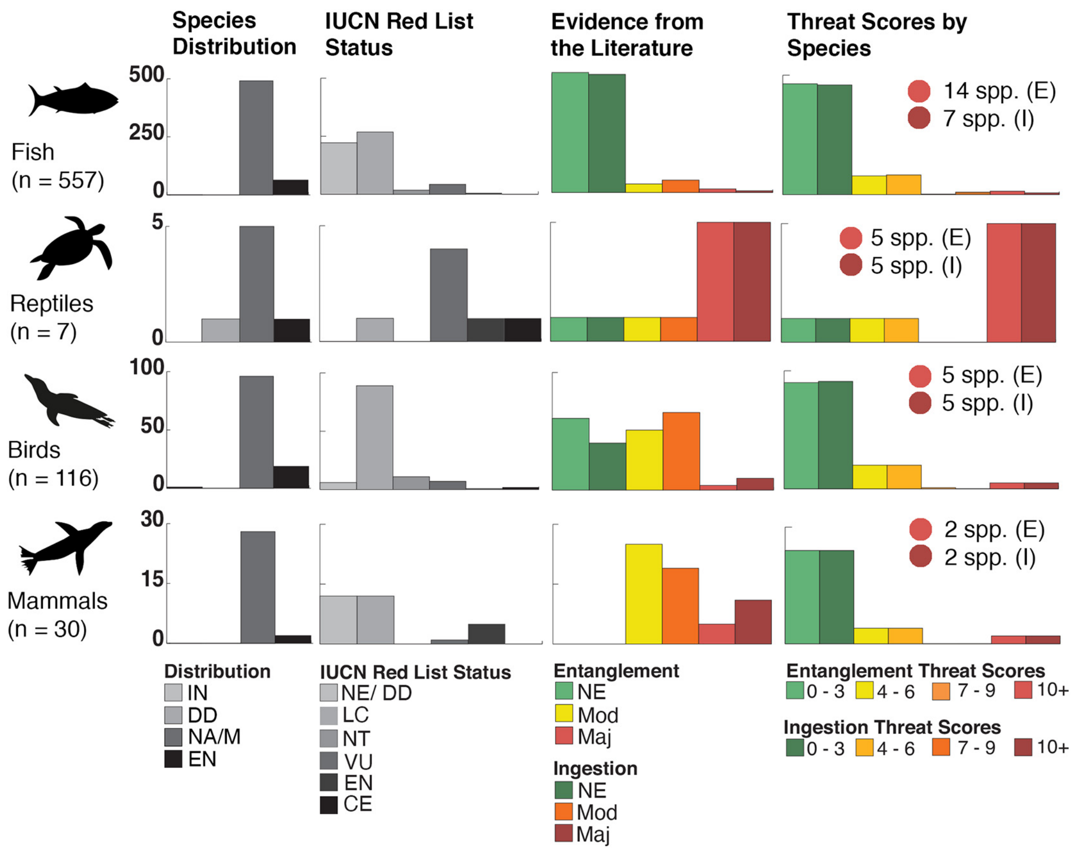

Fig. 6. Summary of priority scoring analysis for Galapagos marine species and plastic contamination. Scoring elements include species distribution, IUCN Red List status and evidence from the literature for harm from plastic contamination caused by entanglement and ingestion at a taxonomic family level. Each element was scored (0–3) and combined to give a final priority score, shown distributed across species within each group in the final column. As numbers in species groups varied from 7 to 557, numbers of species scoring 10+ (severe) for either entanglement and/or ingestion are listed next to red circles for each group resulting in a list of 27 priority species (see Supplementary Table 7 for full species list).

highlighted from our analysis focusing on those found around San Cristobal island.

The highest scoring fish were the iconic scalloped hammerhead (*Sphyrna lewini*) and whale shark (*Rhincodon typus*) (both E = 18, I = 18) due to their conservation status (critically endangered and endangered respectively). Harm caused by entanglement is better understood than the impacts of ingestion in fish, and although increased plastic concentrations associated with ocean frontal systems have been postulated to increase potential uptake for filter-feeders in those regions such as whale sharks and manta rays (Thiel et al., 2018), this cannot yet be linked with predictable harm. Sea turtles are highly vulnerable to interactions with plastic debris and entanglement in derelict fishing gear (Fig. 7b) although data are scarce for the Eastern Pacific region (Nelms et al., 2016). Reptiles are highlighted as the highest priority for investigation of the impacts of plastic contamination in Galapagos (5 out of 7 spp. scored >10; Fig. 6), particularly green turtles (*C. mydas*) and hawksbill turtles (*Eretmochelys imbricata*) (both E = 18, I = 18). Schuyler et al. (2016) predicted that 52% of the global sea turtle population have ingested plastics with consumption of films, fragments, fibres, Styrofoam, sheet-like plastics and bags linked to injury and mortality, with the latter often compared with visual similarities of jellyfish prey. Bags comprised 4% of the total litter items in our study but density varied highly, probably due to in situ fragmentation. At Puerto Tablas, a known turtle foraging area, we collected 107 films and bag fragments from just 100 m of beach, posing a considerable risk if washed back out to sea. Duncan et al. report microplastic (<1 mm) ingestion in 100% of sea turtles analysed, comprising seven species and three ocean basins, suggesting that ingestion of smaller particles could be occurring from the environment, associated with algal food and from trophic transfer from invertebrate prey (Duncan et al., 2019).

Fig. 7. Photographic observations of Galapagos wildlife interacting with plastic items. (a) A Galapagos sea lion (*Zalophus wollebaeki*) with plastic sheeting wrapped around its neck (credit: Juan Pablo Muñoz-Pérez); (b) a green sea turtle (*Chelonia mydas*) entangled in fishing net (credit: Manuel Yépez-Revelo); (c) a flightless cormorant (*Phalacrocorax harrisi*) on its nest including many plastic items, predominantly ropes (credit: Catherine Hobbs).

Due to the lack of familial counterparts, the marine iguana (*A. cristatus*) (E = 12, I = 12) is considered to have a comparable risk to green turtles as both are primarily algae eaters, spend time at the sea surface increasing potential encounter rate with floating plastics and nest in similar beach habitats. On San Cristobal, a new marine iguana subspecies has been recently described at one of our most polluted sites, Punta Pitt (*A. cristatus godzilla*). This subspecies is a major conservation priority due to the very small population size of <500 individuals and high predation pressure from feral cats (MacLeod et al., 2020). The additional potential stress from plastic contamination is therefore of high concern particularly when considering the sensitivity of this species to other pollutants (Wikelski et al., 2002).

Galapagos hosts the world's largest breeding colony of the critically endangered waved albatross (*Phoebastria irrorata*) (E = 18, I = 18) and Galapagos petrel (*Pterodroma phaeopygia*) (E = 18, I = 18), both species known to forage in the Humboldt Current System at increased risk of encounter with floating plastics and at risk of bycatch in fishing grounds outside of the protection of the marine reserve. In addition to the risk of injury for the ingesting adult, there are intergenerational risks from passing plastics to offspring (Ryan, 2015). The Galapagos penguin (*Spheniscus mendiculus*) scored highly (E = 18, I = 18), with evidence from the closely related Magellanic penguin (*S. magellanicus*) where 15% of stranded birds (n = 175) had ingested plastic with one incidence of stomach perforation by a straw (Brandão et al., 2011). Threat of entanglement is high for penguins and the flightless cormorant (*Phalacrocorax harrisi*) (E = 18, I = 12), with most interactions of similar species with fishing lines (Donnelly-Greenan et al., 2019). Integration of plastic debris into nests (Fig. 7c) could introduce entanglement and chemical threats, although direct harm has not been quantified. The majority of the populations of these species are in the west of the Galapagos Archipelago however (Vargas et al., 2005; Ruiz and Wolff, 2011), suggesting that environmental contamination of plastics in these habitats needs to be measured to assess exposure risk.

Only the Galapagos sea lion had published evidence for harmful interactions with plastic within Galapagos, with 251 entanglement incidences recorded between 1995 and 2003, 54% linked to fishery litter and 46% to other litter such as packaging straps, most of which were recorded around the harbour in San Cristobal (see Fig. 7a) (Alava and Salazar, 2006). Therefore, this species is the highest scoring species in our analysis (E = 27, I = 18) and the Galapagos fur seal (*Arctocephalus galapagoensis*) are the Pinnipeds are often seen as sentinels for environmental contamination and have been identified as species of interest for potential biomagnification of POPs (Alava and Ross, 2018). The Galapagos fur seal is found primarily in the west of the Archipelago, again highlighting the urgent need to sample plastic contamination in this ecologically sensitive part of the marine reserve that hosts the highest concentration of endemic marine species (Edgar et al., 2004b).

Our novel rapid assessment tool provides a qualitative way of highlighting priority vertebrate species for plastics research based on the global evidence base. This could support plastic contamination risk mitigation for species and presents a method that could be applied to other vulnerable systems. Although biased by the most studied taxa i.e. coastal species or those that are likely to beach following injury or mortality, this method highlights range-restricted species that are vulnerable to a suite of known conservation threats via the proxy of IUCN Red List data. In addition to highlighting species in Galapagos that are of highest concern, it also highlights the lack of data for many species groups, particularly for fish and invertebrates the latter which were not included due to lack of information on endemism, conservation status and plastic harm impacts. There are measures to address this gap in data that may be useful but require significantly more input from researchers in the region such as using the Marine Biotic Index (AMBI) (Borja et al., 2000) developed to assess benthic ecological quality and consider the sensitivity of different species to disturbance.

Our scoring prioritises conservation as opposed to other considerations such as commercial importance, meaning that cosmopolitan species such as the Yellowfin tuna score low in this analysis. The addition of a commercial importance score could be an important future addition to explore potential links with the human food chain. In benthic invertebrates, the commercially valuable red spiny lobster (*Panulirus penicillatus*) and Galapagos slipper lobster (*Scyllarides astori*) have not been investigated and crustacea have been shown to reduce food consumption and therefore scope for growth due to the false satiation occurring from the ingestion of microplastic fibres in particular. Studies have shown that crustacean guts grind their contents and this creates balls of fibres that may cause blockages (Watts et al., 2014; Welden and Cowie, 2016). Whilst these are not endangered nor endemic, concern must also be paid to commercial species to ensure both food security and economic stability.

4. Conclusions and recommendations

Our findings support the modelled predictions that the Humboldt Current could be a major driver for the rate and spatial distribution of plastic accumulation in this part of the Galapagos Marine Reserve. The apparent connectivity with continental waste streams and fisheries highlights the need for a regional approach in the Eastern Pacific to: (i) assess the sources and pathways of contamination; (ii) evaluate ecological and socioeconomic impacts and (iii) work towards mitigation initiatives at an effective scale. Our data suggest that fragmentation of plastic items may take place in situ on beaches in Galapagos underlining the need for continued clean-up to reduce risks for wildlife and reduce future generation of microplastics. However, this is expensive financially, in terms of carbon footprint and by way of waste management infrastructure requirements that are already over-burdened. Furthermore, more detailed understanding of the relative dynamics of how microplastics make their way into each environmental compartment (sediment, beach, seawater etc.) and how they move around is needed to be able to undertake very localised risk assessments of unexplored locations. Fine detail models will help with this, but also an understanding of the physiographic processes that determine where microplastics end up will support conservation management enabling rapid assessment of localities by simply being able to identify likely beach hotspots for microplastic accumulation and therefore remediation.

Levels of plastic contamination reported here are likely an underestimate due to methodological limitations and the difficulty of accurate polymer identification, particularly for smaller particles and those extracted from organisms. We observed significant accumulations of plastics on rocky lava shores, in mangroves and associated with back-beach vegetation highlighting the need to quantify these temporary sinks that represent key habitats for marine species. We acknowledge that there is a wider suite of risks from plastic contamination than solely ingestion and entanglement that were considered in the priority scoring analysis. Plastic debris acts as a new substrate for rafting organisms (Goldstein et al., 2014), of particular concern in Galapagos with marine ecosystems highly vulnerable to non-native species invasions (Toral-Granda et al., 2017). Galapagos ecosystems are highly impacted by the El Niño Southern Oscillation causing past ecological cascades and regime shifts (Edgar et al., 2010). The multi-stressor effect of warming, food limitation and heightened disease risk could be further exacerbated by plastics and other pollutants in the environment. Several high-risk marine species in our assessment including marine iguanas and Galapagos penguins are already heavily compromised during these climatic fluctuations (Ruiz and Wolff, 2011).

To improve the outlook for the marine wildlife of Galapagos, we recommend: (i) the extension of plastic and ecological surveys around the Archipelago to incorporate further important habitats for priority species and investigate seasonal and inter-annual variation; (ii) the refinement of oceanographic modelling to establish more detailed plastic pathways at a finer scale; (iii) focused investigation on key species to define risks and design interventions; (iv) the development of tools such as predictive models or databases e.g. for strandings to inform mitigation and (v) action higher up the 'plastics chain' closer to source, echoing calls for a coordinated approach to improve waste management strategies across Latin America (Margallo et al., 2019) and in international fisheries operating in the Eastern Pacific (Richardson et al., 2017).

CRediT authorship contribution statement

Jen S. Jones: Investigation, Writing – original draft, Visualization, Writing – review & editing. **Adam Porter:** Investigation, Writing – original draft, Writing – review & editing. **Juan Pablo Muñoz-Pérez:** Investigation, Resources, Writing – review & editing. **Daniela Alarcón-Ruales:** Investigation, Resources, Writing – review & editing. **Tamara S. Galloway:** Methodology, Formal analysis, Writing – original draft, Writing – review & editing. **Brendan J. Godley:** Methodology, Formal analysis, Writing – original draft, Writing – review & editing. **David Santillo:** Formal analysis, Writing – review & editing. **Jessica Vagg:** Formal analysis, Writing – review & editing. **Ceri Lewis:** Investigation, Writing – original draft, Writing – review & editing.

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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