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Sea surface microplastics in the Galapagos: Grab samples reveal high concentrations of particles <200 μm in size

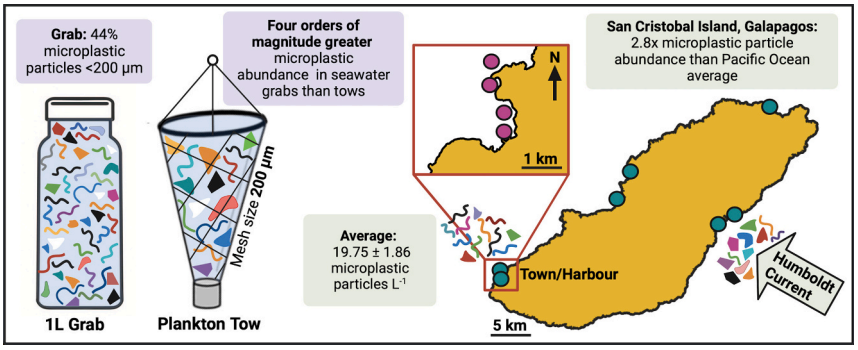
Katie Deakin [a], Georgie Savage [a], Jen S. Jones [a,b], Adam Porter [a], Juan Pablo Muñoz-Pérez [c,d], David Santillo [e], Ceri Lewis [a,*]

[a] Biosciences, Faculty of Health and Life Sciences, University of Exeter, Exeter EX4 4QD, UK
[b] Galapagos Conservation Trust, 7-14 Great Dover Street, London SE1 4YR, UK
[c] Colegio de Ciencias Biológicas y Ambientales (COCIBA), Universidad San Francisco de Quito USFQ, Quito, Ecuador
[d] School of Science, Technology and Engineering, University of the Sunshine Coast UniSC, Hervey Bay, QLD, Australia
[e] Greenpeace Research Laboratories, School of Biosciences, Innovation Centre Phase 2, University of Exeter, Exeter EX4 4RN, UK

HIGHLIGHTS

- Grabs reveal four-orders of magnitude greater microplastic abundances than tows.
- 44 % of microplastic particles in seawater grabs were <200 μm.
- Plankton tows (mesh size 200 μm) underestimate seawater microplastic concentrations.
- Microplastic abundances in Galapagos are 2.8-fold higher than Pacific Ocean.
- Microplastic particle characteristics differ east to west in Galapagos.

GRAPHICAL ABSTRACT



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ABSTRACT

Plastic pollution in the oceans is increasing, yet most global sea surface data is collected using plankton nets which limits our knowledge of the smaller and more bioaccessible size fraction of microplastics (<5 mm). We sampled the biodiverse coastal waters of the Galapagos Island of San Cristobal, comparing two different microplastic sampling methodologies; 1 l whole seawater grab samples filtered to 1.2 μm and sea surface plankton tows with a net mesh size of 200 μm. Our data reveal high concentrations of microplastics in Galapagos coastal waters surrounding the urban area, averaging 11.5 ± 1.48 particles l^{-1}, with a four-order of magnitude increase in microplastic abundance observed using grab sampling compared with 200 μm plankton nets. This increase was greater when including anthropogenic cellulose particles, averaging 19.8 ± 1.86 particles l^{-1}. Microplastic and anthropogenic cellulose particles smaller than 200 μm comprised 44 % of the particles from grab samples, suggesting previous estimates of microplastic pollution based on plankton nets likely miss and therefore underestimate these smaller particles. The particle characteristics and distribution of these smaller particles points strongly to a local input of cellulosic fibres in addition to the microplastic particles transported longer distances via the Humbolt current found across the surface seawater of the Galapagos. Improving our

* Corresponding author.
E-mail address: c.n.lewis@exeter.ac.uk (C. Lewis).

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understanding of particle characteristics and distributions to highlight likely local sources will facilitate the development of local mitigation and management plans to reduce the input and impacts of microplastics to marine species, not just in the Galapagos but globally.

1. Introduction

Mismanaged plastic waste is a worldwide concern, threatening marine habitats and causing physical and chemical pollution on a global scale (Jambeck et al., 2015; United Nations Environment Programme, 2021). This is evident in Ecuador and Peru where mismanaged plastic waste is predicted to increase by ≈184 % between 2010 and 2025 resulting in ≈558,000 t of mismanaged plastic annually (Jambeck et al., 2015). Despite its remote location 1000 km from the coast of Ecuador, and its highly protected status, plastic pollution has been found on the shores, in the surface waters and within marine animals of the Galapagos Marine Reserve (Jones et al., 2021; Jones et al., 2022; Muñoz-Pérez et al., 2023; Sánchez-García and Sanz-Lázaro, 2023), a UNESCO World Heritage Site famous for its unique biodiversity. Recent modelling studies suggest that mainland Ecuador and Peru are a major source of plastic pollution to the Eastern Pacific Ocean, which is then transported by the Humboldt Current from the continental coast to the Galapagos Archipelago, arriving within as little as two months (Van Sebille et al., 2019). This is reflected in beach macroplastic surveys that report higher accumulations of larger marine debris on eastern facing beaches exposed to the Humboldt current, than on the more sheltered western shores (Jones et al., 2021; Muñoz-Pérez et al., 2023). Many Galapagos marine species are already considered to be vulnerable, with 22 marine species in the Galapagos categorised as endangered on the ICUN Red List (Alava et al., 2023). There is therefore concern over the potential additional threats that plastic pollution may pose to Galapagos species through physical harm, life cycle alterations and as a vector for pathogens, invasive species and chemicals (Duncan et al., 2017; Parton et al., 2019; Amaral-Zettler et al., 2020; Muñoz-Pérez et al., 2023).

Previous data collected in 2018 for the most easterly Galapagos Island of San Cristobal using plankton nets (200 μm mesh size) towed at the sea surface, revealed microplastics present in surface seawater at a mean concentration of 0.16 ± 0.03 particles m^{-3} (Jones et al., 2021). The pattern of higher macroplastic concentrations on eastern-facing beaches compared to those on the western side of the island seen in Jones et al. (2021) for beach plastics was not mirrored in the sea surface microplastic data. However, significantly higher microplastic concentrations of 0.89 particles m^{-3} were observed in seawater samples from within the Puerto Baquerizo Moreno harbour, compared with other sites around the island (Jones et al., 2021). This localised hotspot of sea surface microplastics cannot easily be explained by the modelling study highlighting mainland South America as the main source of plastic pollution to the Galapagos (Van Sebille et al., 2019). Industrial fishing fleets, located outside the protected Galapagos Marine Reserve, have also been identified by modelling and macroplastic identification as a mobile source of larger plastic debris to the Galapagos (Van Sebille et al., 2019; Jones et al., 2021). However, the elevated levels of floating microplastics within the harbour raises questions of the potential for an additional local input into the marine environment. Better understanding this potential point-source of pollution could facilitate local intervention and management strategies which are urgently required.

The majority (approximately 80 %) of studies that have contributed to the global ocean's surface microplastic sampling effort have utilised sea surface tows using plankton or neuston net with mesh sizes ranging from 52 to 947 μm (Conkle et al., 2018; Watkins et al., 2021). These studies reveal a five orders of magnitude difference in sea surface microplastic concentrations across the global oceans, with observed microplastic concentrations ranging from 3.5×10^{-6} particles l^{-1} to 5.1×10^{-1} particles l^{-1} (Hidalgo-Ruz et al., 2012; Jambeck et al., 2015; Van Sebille et al., 2015; Green et al., 2018; Watkins et al., 2021). This surface tow methodology enables high volumes of water to be sampled, providing confidence in data reliability. However, net mesh size, dominated by ≈333 μm (Watkins et al., 2021), limits the size fraction of microplastics sampled quantitatively, allowing smaller particles to pass through the mesh and therefore underestimating concentrations of any smaller particles (Barrows et al., 2017; Prata et al., 2019; Hale et al., 2020; Lv et al., 2021). Due to the fragmentation of plastic particles in the environment, it is widely accepted that the number of particles increases as particles get smaller; Besseling et al. (2019) estimated spherical microplastic fragmentation generates $>10^{14}$ times greater numbers of nanoparticles (<1 μm), and Sorasan et al. (2021) similarly showed 10^4–10^5 microplastics/g were generated through UV irradiation of larger plastic particles (Lenz et al., 2016). Fragmentation of microplastics occurs through abiotic and biotic factors, such as photodegradation, mechanical stress and biodisintegration (Zhang et al., 2021). Through these processes, concentrations of microplastics increase with decreased size. Furthermore, microplastic fragmentation generates low molecular weight species, such as plastic additives, which are leached into the surrounding environment acting to concentrate and carry environmentally persistent organic pollutants, further enhancing toxicological effects of microplastics on organisms (Capolupo et al., 2020; Wang et al., 2020; Yu et al., 2021; Biale et al., 2022; Luo et al., 2022).

The difference that mesh size can make on the number of microplastic particles captured in the same volume of seawater was shown by Lindeque et al. (2020); surface seawater microplastic concentrations from two different locations were both 2.5-fold greater when collected using 100 μm nets compared with 333 μm nets. Other studies have compared microplastic concentrations in samples taken using 1 l whole water bottles filtered to 0.45 μm, to samples collected by trawling with a 335 μm and 300 μm mesh size Neuston net (Barrows et al., 2017; Green et al., 2018) and found a 3- to 4-fold higher microplastic concentration per m^3 of seawater, respectively. Barrows et al. (2017) further found ~3-fold greater proportion of 100–1500 μm sized microplastics in 1 l whole water, or 'grab' samples filtered to 0.45 μm, compared with samples collected using a 335 μm mesh Neuston net. These studies are in concordance with Lindeque et al. (2020), indicating larger mesh size reduces the concentration of microplastics found in seawater, as well as the size of particles, limiting our understanding of the abundances of smaller size fractions of microplastic pollution.

Sampling methodologies that use neuston nets will likely also underestimate anthropogenic fibres; a dominant microplastic shape in the marine environment as their diameters can allow them to escape capture in a net (Chubarenko et al., 2016; Steer et al., 2017). Fibres and microplastics <333 μm are widely predicted to elicit greater negative impacts on marine organisms due to their increased bioavailability, gut retention, and greater surface area-to-volume ratio, resulting in increased sorption of pollutants and exposure to organisms (Velzeboer et al., 2014; Qiao et al., 2019). Qiao et al. (2019) identified microplastic fibres illicit greater organism intestinal damage compared with fragments and beads; this included mucosal damage, increased permeability, inflammation, metabolism disruption and microbiota dysbiosis, potentially adversely affecting organism fitness and survivorship. Smaller particles are also bioavailable to a wider range of marine organisms, with particle size influencing microplastic uptake along with distribution within a biological system and subsequent toxic effects (Wright et al., 2013; Lu et al., 2016; Rehse et al., 2016; Jin et al., 2018; Pirsaheb et al., 2020). For example, Lu et al. (2016) observed size-dependent microplastic distribution in the zebrafish, *Danio rerio*, where 5 μm microplastics were retained in fish gills, gut and liver after

entering the circulatory system, compared to 20 μm microplastics accumulating in fish gills and gut only. Subsequently, net-based sampling methods may not provide important data on ecologically relevant sizes of microplastics or their potential threats to marine organisms.

Our current understanding of the threat of seawater microplastic pollution to Galapagos fauna is based on limited field sampling from one year using the more traditional net-based sampling methods and modelling studies (Van Sebille et al., 2019; Jones et al., 2021). The aims of this study are therefore to develop a more detailed characterisation of the sea surface microplastics around San Cristobal Island utilising a combined sampling approach using 200 μm plankton tows, in combination with a targeted grab sampling campaign for capturing the smaller size fraction of particles within the harbour area and coastline of the town of Puerto Baquerizo Moreno; the only major settlement on the island, where microplastics, especially fibres, are expected to be more abundant (Gaylarde et al., 2021; Napper et al., 2023). There is much debate over terminology in the microplastic literature (e.g. Hartmann et al., 2019). For clarity, we use the term microplastic to include anthropogenic cellulose (cellulose particles combined with pigments or chemical modifications, identifying them as manmade as per Lusher et al. (2020) and Finnegan et al. (2022)) unless specifically separated for analytical purposes when discussing, in particular, the likely influence of wastewater.

2. Methods

2.1. Sampling sites

San Cristobal Island (00°54′5.501 S, 89°36′47.537 W) is in the southeast of the Galapagos Archipelago (Fig. 1A), with its eastern side directly exposed to currents, such as the Humboldt Current travelling from mainland South America, contrasting the leeward, sheltered western coastline (Fig. 1B). The Galapagos receives ~270,000 tourists per year, with many passing through San Cristobal as it is home to one of only two airports within the archipelago (Izurieta et al., 2018; Galapagos Government Council, 2021). Subsequently, the island's multi-use

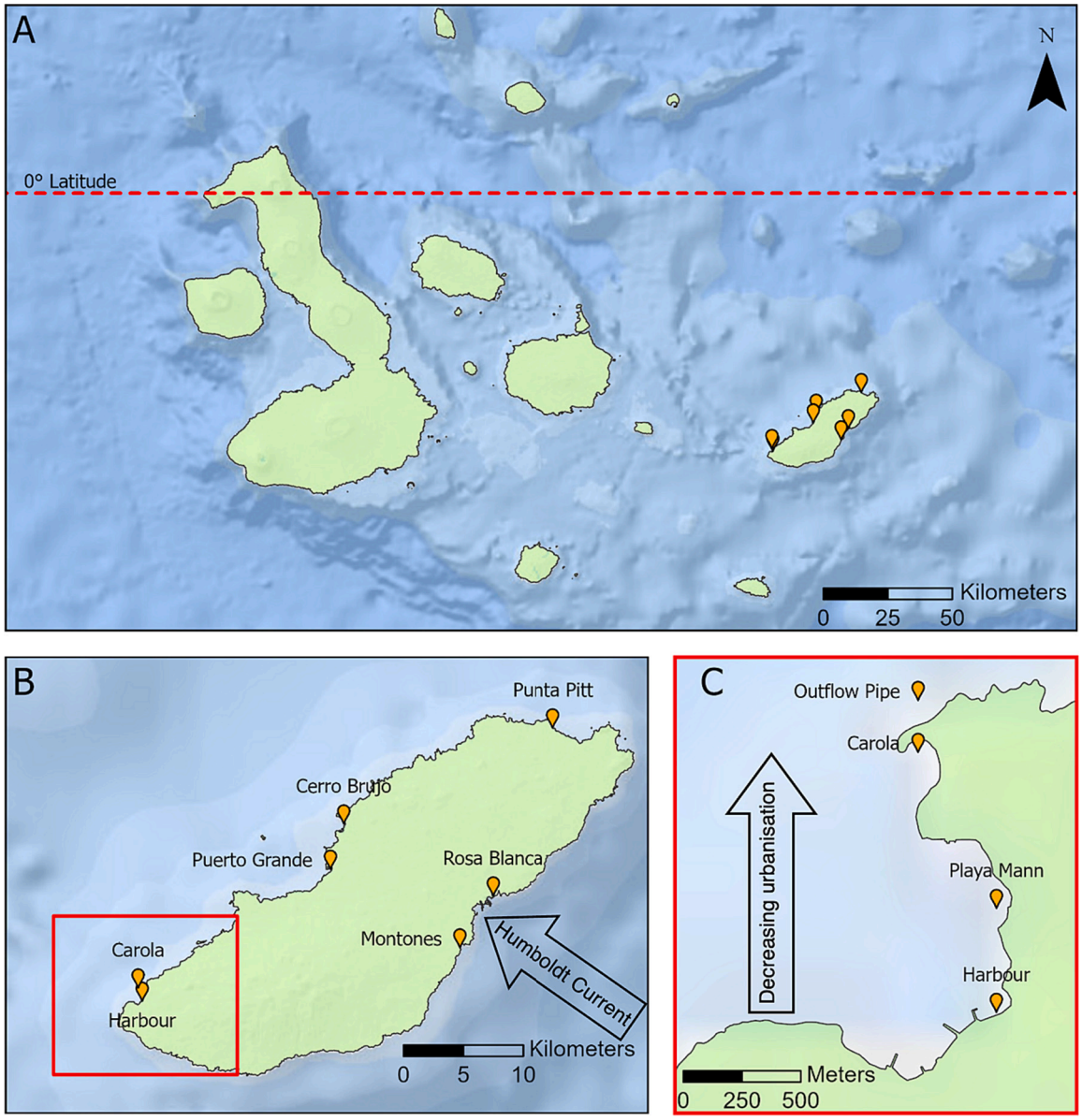

Fig. 1. (A) Geographical location of San Cristobal Island within the Galapagos Archipelago and proximity to the equator, along with the position of (B) plankton tow sites around the island with the Humboldt Currents influence and (C) 1 l grab sites in the bay surrounded by the main town; Puerto Baquerizo-Moreno. Maps were drawn in ArcGIS (ESRI, 2023).

coastline supports intense tourism throughout the year, a harbour town (Puerto Baquerizo Moreno) with ~8000 inhabitants (Fig. 1B), and fishing activity (Izurieta et al., 2018; Galapagos Government Council, 2021). The majority of the island is protected by the National Park resulting in remote areas with restricted access. A sampling campaign was conducted in San Cristobal Island in April 2019.

Nine sites were sampled for sea surface microplastics, seven of which were sampled using plankton tows and are strategically distributed around the island to provide a targeted island wide assessment based on previous work by Jones et al. (2021) (Fig. 1B). From these nine, four sites with decreasing proximity to the town, were sampled using a 1 l whole water 'grab' sampling methodology to determine the impact of urbanisation on seawater microplastic abundance and compare sampling methods (Fig. 1C).

2.2. Sea surface sampling

Three seawater surface tows were performed at each of the seven neuston sites (Fig. 1C). GPS coordinates were recorded at the start and end of each 2–10 min tow, with a boat speed of 0.5–1.5 knots. Tows varied temporally due to varied plankton concentrations and subsequent accumulation within the nets at each site. Tows were undertaken using an unweighted, half-submerged 0.5 m diameter, 200 μm plankton net with a cod end and flow meter attached to enable an accurate calculation of the volume of water sampled. Following each trawl, 0.45 μm filtered seawater was used to concentrate the sample to the cod-end, which was subsequently removed, and contents were poured into a 500 ml Nalgene bottle pre-rinsed with 0.45 μm filtered seawater. In sterile laboratory conditions, samples were dried at 50 °C until only solid matter remained, preparing samples for transport back to the UK.

Samples were then digested to remove the organic matter and isolate the microplastics. Approximately 100 ml of pre-filtered 10 % potassium hydroxide (KOH) solution was added to each sample and heated to 50 °C for 72 h. KOH solution (10 %) was prepared by dissolving 100 g of KOH extra pure pellets (Fisher Scientific) in 1 l of 0.2 μm filtered Milli-Q water. Digestions were repeated for samples with high organic matter content. Samples were vacuum filtered through numerous 70 μm nylon mesh discs; approximately 3 discs per sample due to abundant undigested organic material remaining and these were then sealed in Petri dishes for subsequent analysis. KOH has been shown to degrade polyethylene terephthalate (PET) and reduces the recovery rates of polyvinyl chloride (PVC), suggesting these polymers may be underrepresented within our samples (Dehaut et al., 2016; Karami et al., 2017). Digestion techniques represent a trade-off between the removal of biological matter and the potential degradation of microplastics (Pfeiffer and Fischer, 2020). As such, a 10 % KOH solution at 50 °C was selected to digest organic matter within our samples due to its efficiency, and the relatively low levels of microplastic damage in comparison with other chemicals and temperatures (Dehaut et al., 2016; Karami et al., 2017; Pfeiffer and Fischer, 2020).

Whole seawater 'grab' samples were collected in triplicate from four sites (Fig. 1B). Samples were collected by hand from just below the surface (~20 cm) of the water using 1 l pre-rinsed collapsible water bottles, with GPS recordings taken simultaneously. Vacuum filtration of samples through a 1.2 μm GF/C filter paper followed, and filters were sealed in Petri dishes for later analysis.

Contamination blanks were also collected at all stages in the process and are detailed in the QA/QC section below.

2.3. Particle characteristics/FTIR analysis

Filters from 1 l whole water grab samples and seawater tow samples were systematically inspected using an Olympus MVX10 microscope. Any suspected microplastic particles were quantified and characterised according to shape (fibre, fragment, film, pellet and foam) and colour. Particles were imaged using the attached camera (DP74) and Olympus cellSens Standard Software. Polymer composition was then determined using a PerkinElmer Frontier Fourier-transform infrared (FTIR) spectrometer, using the attenuated total reflection (−ATR) universal diamond attachment for particles >1 mm. Particles <1 mm were picked from the filter papers using micro tweezers and transferred to a diamond compression cell and set to scan on the PerkinElmer Spotlight 200 μFTIR in transmission mode (wavenumber resolution 4 cm^{-1}, 16 scans, range from 4000 to 600 cm^{-1}). Compression cells have an advantage over filter scanning as they squash the particles to enable transmission of the infra-red light through the particle giving more accurate spectra than using reflectance modes, and they ensure the particle is not lost. Spectra were refined using the linear normalisation, base-line correction and data tune-up tools from the Perkin-Elmer's IR Spectrum™ 10 software (version 10.5.4.738). FTIR-generated spectra were compared to industry libraries and accepted using a general 65 % match threshold, accompanied by expert assessment of characteristic spectral peaks. Plastic additives, such as cyanox and varox, have been included in the plastic counts, based on the understanding that due to the degraded nature of these environmental samples, the anthropogenic synthetic polymer is identified instead of the petrochemical polymer (Lusher et al., 2020). Further, we include anthropogenically modified cellulosic fibres in our definition of 'microplastic' as they have undergone chemical alteration and extrusion similar to synthetic fibres (Athey and Erdle, 2022), and are a major component of anthropogenic debris worldwide (Adams et al., 2021; Napper et al., 2021).

The polymer composition of all the particles identified in the 1 l whole water grab samples ($n = 308$) was determined and subsequently measured using Image J software, recording feret diameter, minimum feret and area (9.8 % of grab particles could not be measured due to poor image quality). Due to the vast quantity of particles isolated from the seawater tow samples ($n = 4744$), a stratified and randomized approach was developed; 10 % or a minimum of 3 particles (highest value) within each combined colour and shape category were imaged, measured and polymer composition determined, resulting in 26.7 % of particles being analysed by FTIR ($n = 1265$).

To convert microplastic counts for each tow into abundance per m^3, the total particle counts were corrected for contamination (using procedural blanks from the field and the laboratory) and polymer attribution (FTIR data). Firstly any shape x colour combination within a tow found to have an FTIR match score of <65 % was removed. Subsequently, abundances were corrected for contamination by removing particles that matched those in the blanks (see detail in QA/QC below) and this abundance value was then divided by the volume of water sampled (calculated using flow rate data). To compare tows to the grab samples, microplastic abundance per m^3 for each sample was determined by multiplying FTIR confirmed counts by 1000 to convert l to m^3.

2.4. QA/QC

To minimize and quantify any inadvertent contamination from the air or sampling equipment the plankton net was rinsed between replicates and a damp GF/C filter paper in a Petri dish was positioned at the net opening or near sample collection. A procedural blank was undertaken in the field to account for any contamination within the net or air; the net was suspended above the sea surface for 'tow' duration and subsequently rinsed using filtered seawater, into a sample bottle for later laboratory processing. Similarly, a blank sample was undertaken for the grab samples by filling a container with filtered seawater for subsequent laboratory processing.

Procedural blanks were undertaken for all sampling types to ensure microplastic contamination during laboratory processing of samples was accounted for; this was achieved by performing laboratory processing on an 'empty sample' using the same chemicals and laboratory consumables utilised for processing field samples. Sterile plastic consumables were immediately used after opening and all other laboratory equipment was rinsed three times using MilliQ before each processing stage.

Where possible, consumable lids were used and aluminium foil caps on equipment were functioned to prevent airborne contamination. All chemicals used were filtered through 0.2 μm Nalgene filter cups in a laminar flow hood prior to use. Any processing of samples, such as digestions and vacuum filtration, were undertaken in a laminar flow hood to reduce airborne contamination. Airborne contamination was controlled for throughout sample processing and analysis by leaving a damp GF/C filter paper in a Petri dish in close proximity to the exposed sample. Airborne contamination blanks and procedural blanks were inspected for microplastics using FTIR (as described above). Particles were classified according to combined particle shape, colour, and polymer. The specific particles were subtracted from the respective data per site and sample before further analysis and all data presented, except particle size analysis to eliminate bias, has been corrected for the contamination controls. Particle loss during sample processing was determined and taken into consideration when interpreting results but not corrected for in the following data, providing conservative abundances as per Rochman et al. (2019). In brief, 500 × 23 μm nylon fibres and ground nylon fragments (>150um <300um) were stained with nile red (Maes et al., 2017) and known concentrations were mixed into 6 samples of filtered seawater; and 3 processed as net tow samples and 3 as grab samples. Recovered plastics were counted by fluorescent microscopy using an Olympus SZ16 microscope with Reflected Fluorescence Illuminator (SZX2-RFA16) utilising a red fluorescence protein filter cube (SZX2-FRFP1/RFP1; excitation filter BP530–550, barrier filter BA575IF).

2.5. Statistical analysis

All statistical analysis was performed using RStudio (version 2022.07.1 + 554; R Core Team, 2023). Y-axis break in boxplots followed R script 'ggbreak' as per Xu et al. (Xu et al., 2021b). The data from both tow and grab methods were analysed using a linear model to look for differences in microplastic abundances across sites. The residuals of the linear models for both grab and tow data were then tested for normality using a Shapiro-Wilk test. Tow data were not normally distributed ($W = 0.8456$, $p = 0.0036$) so a negative binomial GLM was undertaken to test for significant differences in microplastic abundances between sites due to overdispersion in the data. The grab sample data were normally distributed ($W = 0.961$, $p = 0.801$) so a One-Way ANOVA was used to test for significant differences between sites. Statistical significance was assumed at $p < 0.05$.

3. Results

Across the seven sites, a total of 4744 suspected microplastic particles were isolated from the surface seawater tow samples of which 82.8 % confirmed as microplastic, and 17.3 % as natural or 'other' anthropogenic particles. Grab sampling isolated a total of 308 suspected microplastic particles across four sites, 81.5 % as microplastic, and 18.5 % as natural or 'other' particles.

3.1. Sea surface tows

Microplastic contamination was present at all seven sites with a total of 3926 confirmed microplastic particles collected using a 200 μm plankton net. Mean microplastic concentration was 2.56 ± 0.78 particles m^{-3} across all sites, ranging from 0.61 ± 0.67 particles m^{-3} at Puerto Grande to 10.81 ± 1.56 particles m^{-3} at Montones (Fig. 2). A negative binomial regression revealed that sample site; specifically Montones had a significant influence on microplastic abundance (Estimate = [1.947843], SE = [0.507863], z = [3.835], p = [0.0001]). Montones had a 7-fold greater abundance of microplastic compared to the next most contaminated site; the Harbour (Fig. 2).

Of those microplastics, anthropogenic cellulose comprised a mean of 0.16 ± 0.06 particles m^{-3} for all study sites. These anthropogenic cellulose concentrations ranged from 0.03 ± 0.027 particles m^{-3} at Carola to 0.46 ± 0.077 particles m^{-3} at Montones.

3.2. Grab sampling

Grab samples contained 251 confirmed microplastic particles present in the surface seawater samples from all four sites on San Cristobal Island, with a mean of 19.7 ± 1.9 particles per litre (19,750 ± 1855.07 particles m^{-3} when scaled up). Microplastic concentrations ranged from 13.6 ± 3.18 to 23.3 ± 4.67 particles per litre (13,666.67 ± 3179.80 particles m^{-3} to 23,333.33 ± 4666.67 particles m^{-3} when scaled up) at Carola and the Outflow Pipe respectively, with no significant difference

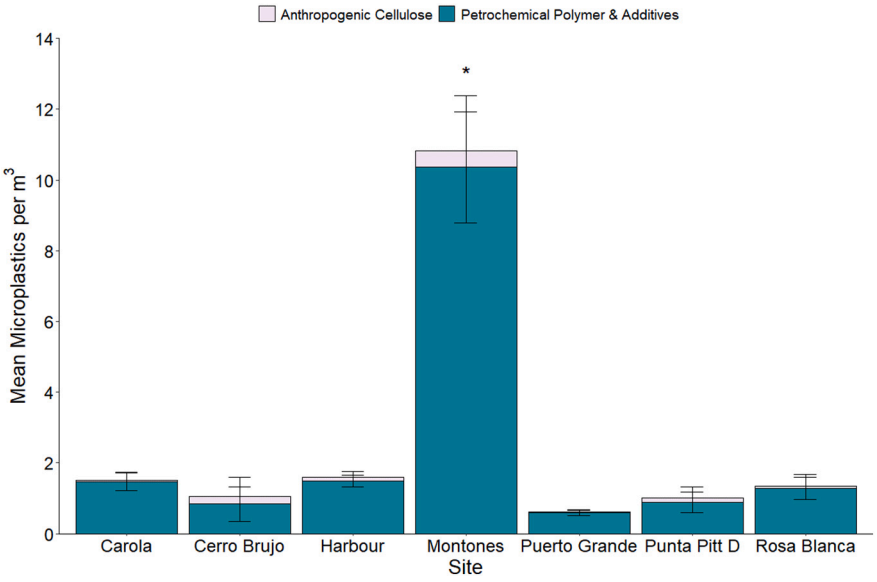

Fig. 2. Mean abundance of microplastic particles ($n = 3926$) for sea surface water samples at seven sites around San Cristobal Island, Galapagos, collected using 200 μm plankton tows. Microplastic particles are divided into two categories; anthropogenic cellulose particles, and petrochemical polymer particles & additives, indicating the high prevalence of the latter at all sites. Error bars indicate standard error of petrochemical & additive means as well as total microplastic polymer mean abundance for each site. The asterisk over Montones denotes the significantly increased microplastic abundance at this site from all other sites.

between sites (Fig. 3; One-Way ANOVA, $F(3, 8) = 1.7977$; $p = 0.226$).

Of those microplastics, anthropogenic cellulose comprised a mean of 8.25 ± 1.59 particles l^{-1} for all study sites. These concentrations ranged from 5.0 ± 1.73 particles l^{-1} at Playa Mann to 12.3 ± 4.84 particles l^{-1} at the Outflow Pipe; equivalent to a range of roughly 5000 to 12,300 particles per m^{-3}.

3.3. Contamination

Airborne contamination was recorded at all sampling sites with a mean of 7.9 ± 7.6 microplastic and fibres represented 68 % of these particles. Contamination in the laboratory was also present with 14 fibres and 17 fragments in total being recorded on the damp GF/C filter paper during the filtration, microscopy and FTIR processing of all samples combined. Sample processing in the lab revealed a microplastic recovery rate of 69 % and 77 % in tow sample methodologies for fibres and fragments respectively, and 100 % for grab sample methodology. Data here are reported without correcting for particle loss during laboratory processing as per Rochman et al. hence data are conservative (Rochman et al., 2019).

3.4. Size distribution of sea surface microplastic particles

All confirmed microplastic particles collected from across the 7 tow sites were measured ($n = 1153$). Microplastic particles between 1000 and 1999 μm accounted for 20.0 % of all particles (Fig. 4A). Particles in tow samples greatly varied in size with generally larger particles recorded; size categories 300–399 μm and 2000–2999 μm represented 10.1 % and 9.3 % of particles, respectively (Fig. 4A). Across the 4 sites where grab sampling was employed, confirmed microplastic particles were measured ($n = 240$), where possible, revealing the majority of particles to be between the smaller size classes of 100–199 μm and 50–99 μm, 24.2 % and 20.0 %, respectively (Fig. 4B). Our lower limit of detection is set by particles that can be picked from a filter paper to be analysed by FTIR at 50 μm. A general decrease in the abundance of microplastic particles smaller than 100–199 μm is then observed; however, microplastic particles between 1000 and 1999 μm compromised 6.7 % of all microplastic, showing a peak at this size category similar to tow samples (Fig. 4B).

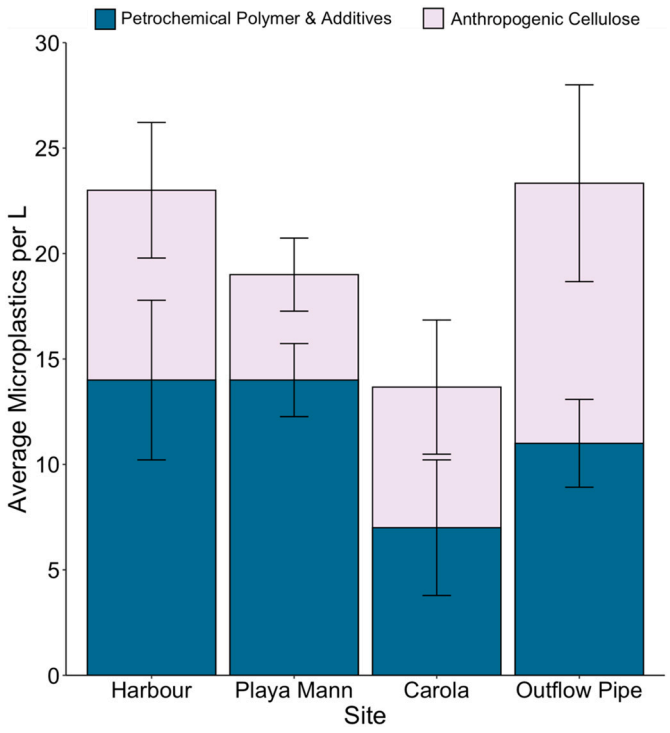

Fig. 3. Abundance of microplastics in surface seawater around Puerto Baquerizo Moreno, San Cristobal Island, Galapagos, sampled using Grab methodology. Stacked bar chart of mean microplastic concentrations ($n = 251$) from four sites on San Cristobal Island with differing proximity to urbanisation; no significant difference between sites was found. Microplastic particles are divided into two categories, petrochemical polymers & additives ($n = 138$) and anthropogenic cellulose ($n = 113$), indicating the high prevalence of the latter at all sites. Error bars indicate standard error of petrochemical & additive means as well as total microplastic polymer mean abundance for each site.

3.5. Spatial distribution of microplastic particle characteristics

Tow microplastic particles comprised mostly fragments (48.1 %), fibres (39.9 %) and films (11.7 %), with shape composition varying across sites. Generally, fibres were the dominant shape at sites along the western coast of San Cristobal, ranging from 35.6 % to 51.7 % at Carola and the Harbour respectively, compared with lower compositions on the eastern coast, ranging from 21.1 % to 47.5 % at Punta Pitt and Rosa Blanca, respectively (Fig. 5A). Similarly, films were often found on the western coast representing 9.9 % to 39.7 % of particles at the Harbour and Cerro Brujo respectively, whereas they comprised only 7.7 % to 7.8 % of particles on the eastern coast, at Montones and Punta Pitt respectively (Fig. 5A). Conversely, fragments represented only 18.1 % to 51.9 % of all microplastic particles at Puerto Grande and Carola on the western coast and were dominate microplastics on the eastern coast ranging from 44.2 % to 70.7 % at Rosa Blanca and Punta Pitt, respectively (Fig. 5A).

Microplastic particles within grab samples consisted of four shape categories; generally, fibres were the most common shape found across all sites representing a mean of 48.7 % and ranged from 40.6 % to 56.3 % at Playa Mann and the Outflow Pipe respectively (Fig. 5B). Fragments were similarly recorded in high numbers representing 35.6 % to 45.9 % of all microplastic particles at the Outflow Pipe and Playa Mann respectively, with a mean across all sites of 40.8 % (Fig. 5B).

FTIR analysis determined the polymer composition of particles in tow samples ($n = 1265$, 26.7 % of total particles) and grab samples ($n = 308$, 100 % of total particles). Particles identified as natural and 'other' anthropogenic particles (17.3 % of tow and 18.5 % of grab particles) have been excluded from polymer composition data. Of the 83.5 % ($n = 1056$) of particles analysed by FTIR that were microplastic; polyethylene and polypropylene were generally the most abundant in tow samples, ranging from 12.1 % to 36.6 % (Fig. 6A vi and i). However, PVC was the most abundant polymer at Puerto Grande (32.5 %), with acrylic similarly abundant at this site (11.7 %), as well as Punta Pitt (16.9 %) (Fig. 6A iv and vi). PVF and plastic additives (classified based on Lusher et al. (2020), including fillers and additives associated with plastic polymer materials, (e.g., plasticizers)) surprisingly represented 17.4 % and 24.9 % of polymers at Montones (Fig. 6A v). Further, 27.7 % of particles at Cerro Brujo were confirmed as anthropogenic cellulose (Fig. 6A ii). Polymer composition in grab samples varied greatly compared to tow samples; anthropogenic cellulose particles were most abundant across all sites, ranging from 26.8 % to 52.9 % of all particles (Fig. 6B ii and iv), with polypropylene representing only 0 % to 4.3 % of particles across all sites (Fig. 6B iii and i). Polyethylene (9.8 % to 23.2 %) and plastic additives (7.3 % to 23.2 %) were also found commonly within grab samples (Fig. 6B iii and i). Anthropogenic cellulose was of particular interest as on mean 81.6 ± 5.1 % of fibres were made of anthropogenic cellulose.

All microplastic particles within tow and grab samples were categorised by colour. Microplastic particles identified in tow samples were generally dominated by black (10.9 %–34.0 %) and blue (5.4 %–25.0 %) particles (Fig. 6A xii and xiii; Fig. 6A xi and x, Fig. 7). Clear microplastic particles compromised the majority of particles at Puerto Grande (60.8 %) and were also prevalent across all sites (Fig. 6A xi). Surprisingly, brown, and white microplastic particles represented the most abundant

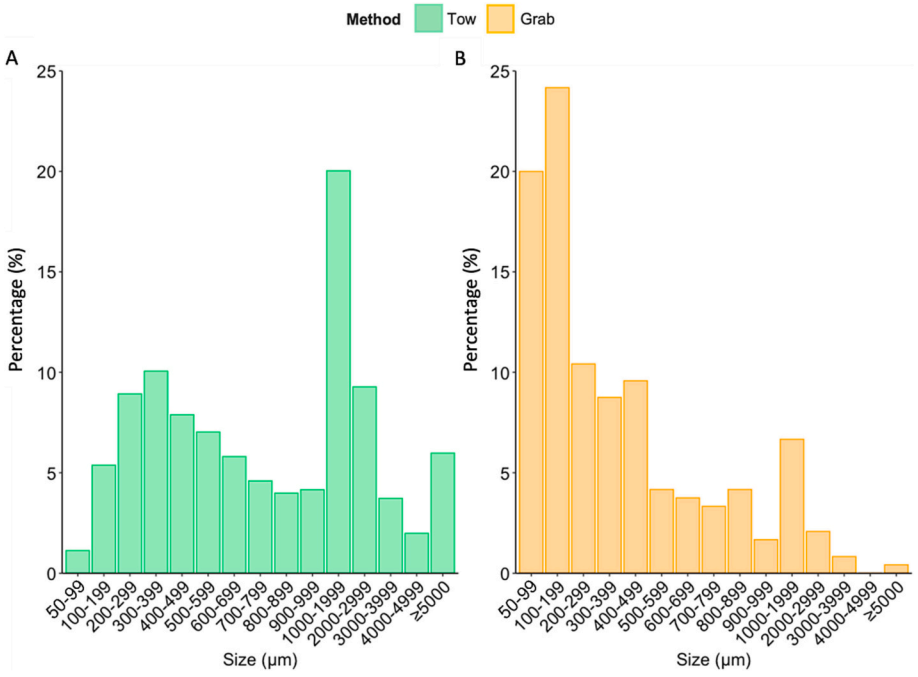

Fig. 4. Size classification of microplastic particles collected from surface seawater samples around San Cristobal Island, Galapagos, collected using Tow and Grab methodologies. Data presented as percentage (%) of total particles assessed to normalise for the different number of particles assessed and the different volumes of seawater taken using the two techniques for (A) Tow Samples (n = 1153) and (B) Grab Samples (n = 240).

colour at Cerro Brujo (37.9 %) and Montones (39.9 %), despite limited composition across sites (Fig. 6A ix and xii). Colours prevalent within grab samples similarly included blue (18.4 %–32.1 %), black (18.7 %–25.0 %) and clear (8.1 %–18.7 %) particles (Fig. 6B v-viii). Red microplastic particles were ubiquitous across the 4 sites, comprising 9.2 %–13.2 % of particles (Fig. 6B v-viii, Fig. 7).

4. Discussion

These data reveal high concentrations of smaller microplastic particles present in the harbour surface seawater of San Cristobal Island in the Galapagos Archipelago, with a mean microplastic concentration of ≈19.7 particles Litre^{-1} (≈19,750 particles m^{-3}). The grab sampling data presented a four order of magnitude increase in microplastic abundance, compared to sea-surface tows using a 200 μm mesh plankton net for the sites where both techniques were used. Data collected using the more traditional surface tows revealed mean microplastic concentrations of 2.42 ± 0.74 particles m^{-3} for San Cristobal Island. These considerable differences in microplastic concentration in seawater when utilising different methodologies suggest that previous estimates of floating microplastic pollution within the Galapagos (0.16 ± 0.03 particles m^{-3} using plankton tows by Jones et al., 2021), have underestimated the true extent of microplastic pollution in these waters, at least for the locations nearest the town. Of the microplastic particles within the grab samples that were measured, just under half (44.2 %) were <200 μm in size and so would be expected to pass through the mesh of a plankton net. The lower limit of detection for grab sampling is likely set by the ability to pick the particle from the filter paper for analysis on the FTIR (~50 μm), rather than the mesh size the water was sampled to, whereas the tows cannot quantitatively sample particles smaller than the 200 μm net mesh size.

The abundance of microplastic particles recorded here for the Galapagos are high and exceed the estimated safe limits for buoyant microplastic particles (estimated as 6650 particles m^{-3}, Everaert et al., 2018). Our microplastic concentrations using grab sampling were over 5-fold greater than that found in Plymouth Sound in the UK (≈2.6 particles l^{-1}), an area of relatively high urbanisation when utilising a similar methodology (Green et al., 2018). Microplastic concentrations collected by grab sampling in a similarly remote archipelago, Ma'an, off the eastern coast of China, had much smaller abundances (0.2–0.6 particles l^{-1}) than our study in Galapagos (Zhang et al., 2020). Ma'an is located within the largest fishery in China, the greatest contributor of mismanaged plastic waste entering the environment globally (Jambeck et al., 2015; Zhang et al., 2020). Using citizen science data collected globally using a standardised grab sample protocol, Barrows et al. (2018) report a global mean of sea surface microplastic concentration of 11.8 ± 0.6 particles l^{-1} (equivalent to 11,800 particles m^{-3}) and an mean microplastic concentration of 7.0 particles l^{-1} (equivalent to 7000 particles m^{-3}) within the Pacific Ocean; abundances from our Galapagos grab samples are greater than these global and ocean specific means with microplastic concentrations averaging 19.8 ± 1.9 particles l^{-1} (equivalent to 19,750 ± 1855 particles m^{-3}). However, using these same data for fibres alone, Lima et al. (2021) estimate by modelling a global mean sea surface concentrations of ≈5900 ± 6800 microfibres m^{-3}; ranging from ≈496 ± 630 microfibres m^{-3} in the Persian Gulf to ≈27,000 ± 18,000 microfibres m^{-3} in the Hudson Bay, USA. The concentrations of microplastic particles measured here therefore were high, but within the predicted global range.

Our samples from sites in the port area of Puerto Baquerizo Moreno ranged from 13,667 ± 3180 particles m^{-3} at Carola; a small beach embayment, to 23,333 ± 4667 particles m^{-3}, at the Outflow Pipe. Using the search engine in Google Maps (Google Maps, 2023) there are six registered laundry services in a ~3 km^2 area (Google Earth, 2023) serving a population of ~6500 people (Jahnke et al., 2021) and the Galapagos islands welcomed 271,238 visitors in 2019 alone (Escobar-Camacho et al., 2021). These large numbers of visitors concentrated in relatively small area (~0.5 % of San Cristobal Islands total land area of 558 km^{-2}; Dvorak et al., 2020) represent a significant burden on the environment. Fibres comprised between 40.5 % and 56.3 % of all particles collected by the 1 l grab samples, greatest at the Outflow Pipe, and of those fibres 70–95 % were anthropogenic cellulose. Anthropogenic cellulose fibres have been linked with the washing of clothes garments (De Falco et al., 2019; Chakraborty et al., 2022), and further, materials flushed down toilets such as toilet paper and wet wipes for which there is

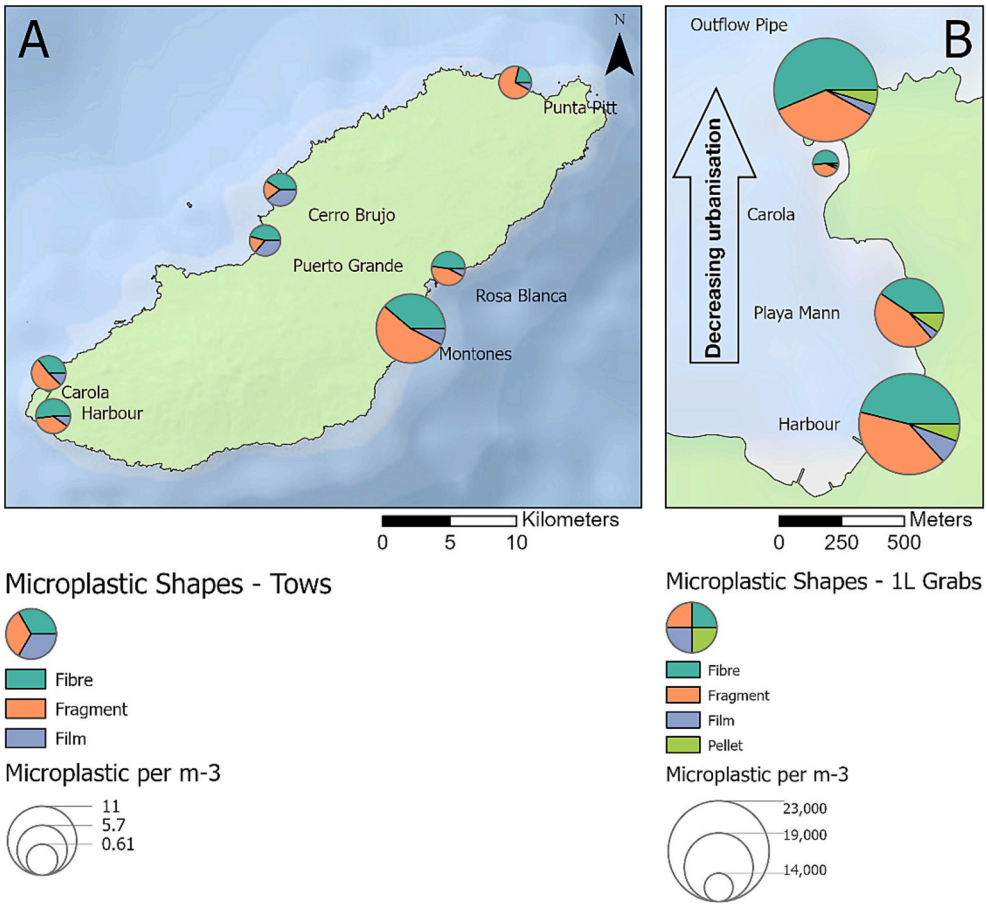

Fig. 5. Spatial distribution of microplastic particle shape composition in surface seawater around San Cristobal Island, Galapagos, using tow and grab sampling techniques. Pies indicate the percentage composition of each shape category at the respective site for (A) tow samples and (B) grab samples. In Panel A, pellets, foams, and fibre bundles were not drawn as these categories could not be resolved graphically due to their very low relative abundance. In Panel B fibre bundles and foams were not present. Maps were drawn in ArcGIS (ESRI, 2023).

no sewage treatment in the Galapagos may contribute to the elevated levels of cellulosic fibres in the harbour area (Napper and Thompson, 2016; Pantoja Munoz et al., 2018; Grbić et al., 2020) indicating the pressures the local population and in particular tourism are exerting in this area.

The efficacy of grab sampling in estimating seawater microplastic abundance has been demonstrated by a number of previous studies. Barrows et al. (2017) and Hung et al. (2021) demonstrated grab sampling collected 1180 and 1352 more microplastics, respectively, than samples collected using 335 μm mesh trawls taken at the same time. Lindeque et al. (2020) compared microplastic abundance data collected using different plankton net mesh sizes and found that the smaller 100 μm mesh measured 10-fold higher concentrations than a 500 μm mesh, as a result of smaller particles escaping the lager mesh. Extrapolating from this data they estimated that if mesh sizes were reduced from 333 μm to 1 μm, enabling smaller particles to be included in sampling campaigns, like grab methods, microplastic abundance could increase 907-fold, providing a more accurate representation of environmental concentrations (Lindeque et al., 2020) and utilising a simpler approach. The differences in the size composition of microplastics collected using the grab and tow samples within our data aligns with this theory; microplastic particles <200 μm dominated grab samples (44.2 %) yet only represented 6.5 % of particles within the sea surface tow samples, where microplastic particles between 1000 and 1999 μm instead dominated the sample (making up 20 % of the particles captured). Whilst some of this variation may be a result of site differences and stochasticity, this noticeable variation in size composition is most likely driven by the sampling methods (Barrows et al., 2017; Green et al., 2018; Garcia et al., 2020; Lindeque et al., 2020; Schönlau et al., 2020; Watkins et al., 2021) and this points to previously published estimates for microplastic abundances in Galapagos seawater as likely missing this smaller fraction of microplastic particles.

In addition to grab samples capturing greater microplastic abundances and smaller particle sizes than the tow samples, they also captured different particle shapes, with tow sampling comprising mostly fragments (with a mean of 48.1 % across all sites), compared with fibres dominating grab sampling (mean of 48.7 %). Fibres are less likely to be retained in a 200 μm mesh under the water pressure exerted during a tow due to their small diameter, resulting in lower concentrations (Barrows et al., 2017; Schönlau et al., 2020; Watkins et al., 2021). Our data also reveal a large variation between replicates at the same site when using grab sampling compared to tow sampling, similar to that observed in several other studies (Barrows et al., 2018; Zhang et al., 2020; Hung et al., 2021; Watkins et al., 2021). For example, at our Carola sampling site, microplastic concentrations ranged from 8 to 19 particles l^{-1}. This heterogeneity may be a result of microplastic aggregations around biological material in these highly productive waters or the local currents and eddies that are not accounted for when sampling small volumes; the small volume sampled, compared to tows, may magnify this variability within the surface seawater and even the kinetics of the sampling method may aggregate plastics floating on the surface due to the drawdown of water into the sampling vessel. Surficial deposition of particularly microfibres has also been shown to potentially be a greater vector of microfibres to surface waters than emissions from washing garments (Napper et al., 2023) and given their large aspect ratios and the surface tension of water, they may accumulate on the

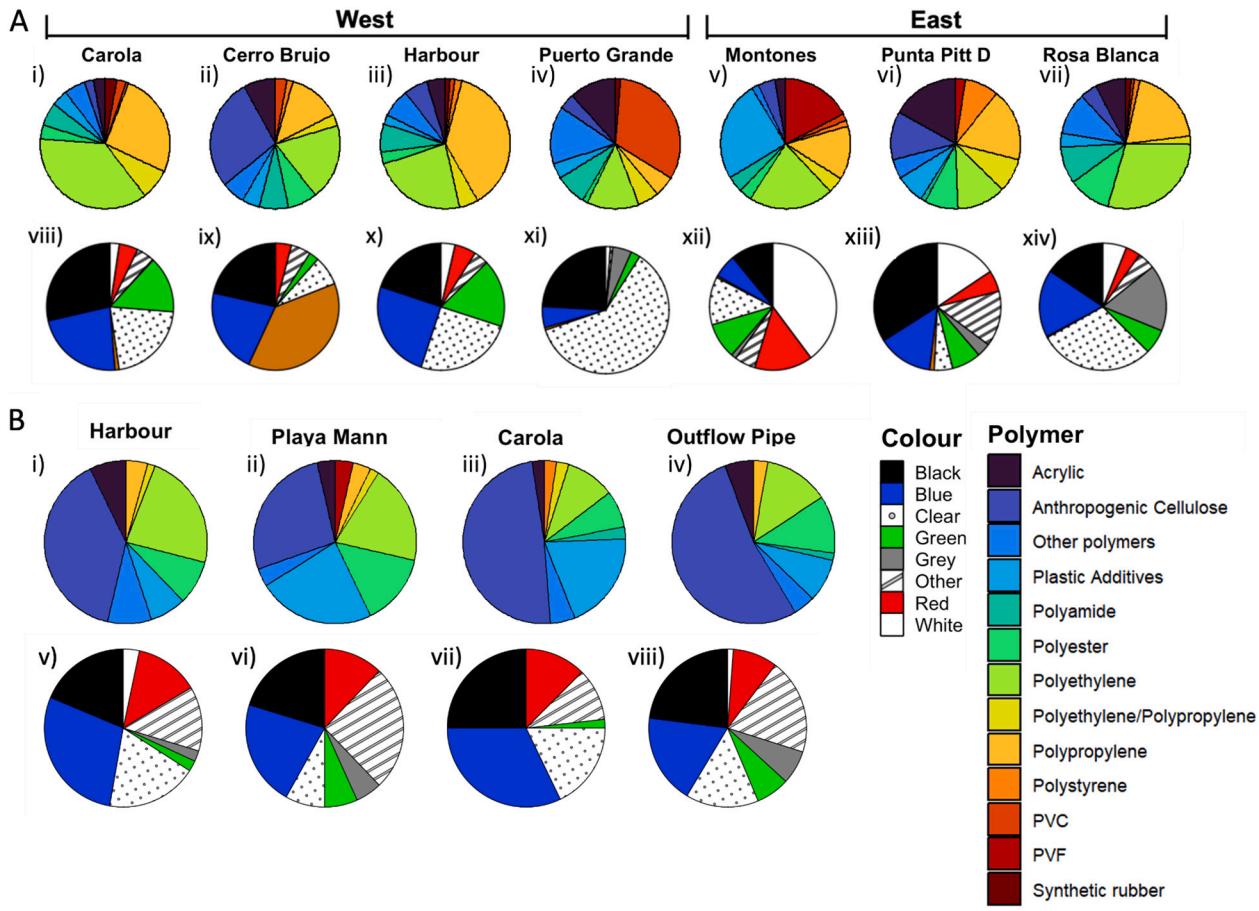

Fig. 6. Microplastic polymer and colour composition of surface seawater tow and grab samples, San Cristobal Island, Galapagos. Pie charts indicate the percentage composition of polymers and shapes at the respective site for (A) tow samples and (B) grab samples. (A) 26.7 % (n = 1265) of all suspected microplastic particles in tow samples were analysed by FTIR, identifying polymer composition; of these 82.8 % were confirmed as plastic (i-vii). All microplastic particles across sites were categorised by colour and are represented by figures viii-xiv. (B) Polymer composition of all particles in grab samples were identified (n = 308); of these 81.2 % of particles were confirmed as plastic or anthropogenic cellulose (i-iv). Colour composition of all microplastic particles across sites are shown in figures v-viii.

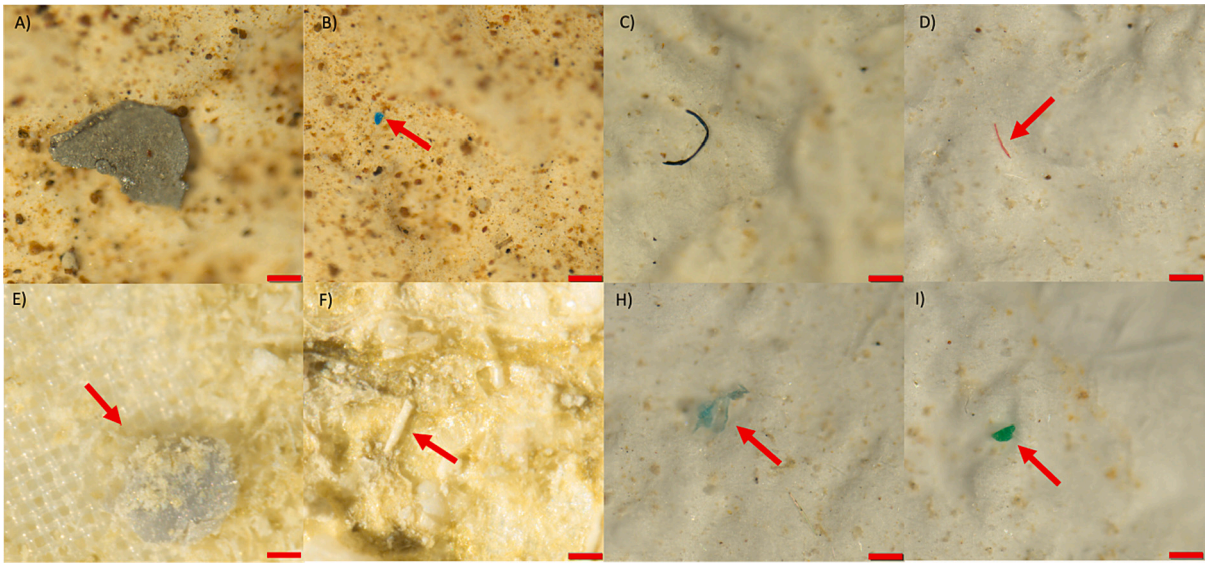

Fig. 7. Example images of microplastic particles found within seawater samples, collected using grab and tow techniques, around San Cristobal Island, Galapagos. Red scale bars represent 200 μm. Particles indicated by red arrows include a (A) grey acrylic fragment, (B) blue polyester fragment, (C) black anthropogenic cellulose fibre, (D) red plastic additive fibre, (E) grey PVC fragment, (F) white PVF fibre, (G) blue polyethylene film and (H) green polypropylene/polyethylene fragment.

surface in proximity to human settlements. To increase the reliability of this method for any long-term monitoring applications, a greater number of replicates, larger volume grabs samples as well as greater spatial area sampled per site may be required to account for this variability in localised concentrations as well as some careful investigations over the mechanics of the sampling method itself. Our data are a snapshot in time; spatiotemporal sampling campaigns considering factors such as seasonal variation, weather, and targeted, high resolution sampling campaigns around point sources such as the outfall pipe are required to fully understand local plastic input into the marine environment.

Despite these limitations, processing grab samples within the lab resulted in a 100 % recovery of microplastics, compared with 69 % of fibres and 77 % of fragments recovered from the tow sample processing method. This microplastic loss was a result of high organic (planktonic) matter within the tow samples, requiring chemical digestions to enable microplastic isolation, perhaps also contributing to some of the differences in shape composition between the tow and grab samples that were observed and the potential for masking of particles by organic matter after digestions, even after careful microscopy. Whilst we did quantify a higher microplastic particle loss during sample processing in the laboratory for the tow samples, this is not enough to account for this four-order difference in microplastic abundance between sampling methods.

Understanding the smaller fraction of microplastic pollution provides important information for understanding the potential impacts on marine species; smaller particles have a stronger sorption capacity for hydrophobic pollutants (Devriese et al., 2017) and will be bioaccessible to a much wider range of marine species for uptake via ingestion, having the potential to elicit greater harm to biota and their ecosystem functionality (Wright et al., 2013; Lu et al., 2016; Rehse et al., 2016; Galloway et al., 2017; Jin et al., 2018; Zhao et al., 2018; Pirsaheb et al., 2020). Studies have also identified the ingestion of microplastics in marine species, such as *Mytilus coruscus* and *Mytilus edulis*, to be negatively size dependent, with smaller microplastics spending a greater period of time in digestive tracts (Scott et al., 2019; Wang et al., 2021). Microplastics have already been documented within marine species in the Galapagos and a number of species have been identified to be at high risk from the impacts of plastic pollution (Jones et al., 2021). Subsequently, characterising the smaller fraction of microplastics within Galapagos seawater would provide further insight into the environmental exposure and risk to these marine organisms.

Whilst grab sampling enables the smaller fraction of microplastic pollution to be assessed, this methodology analyses much smaller volumes of water and so perhaps limits spatial assessments of patterns of microplastic abundances at larger island or regional scales. The data from our plankton tows, which sampled much larger volumes of water (mean 93.29 $\pm$ 13.88 m^{-3}) over greater distances, reveal patterns in particle morphology of the microplastic pollution around San Cristobal Island from which some inferences about sources might be made (Rochman et al., 2019). We observed different microplastic particle morphologies along the island's east and west coastlines; fragments represented over half of particles across sites on the east, reaching 70.7 % at Punta Pitt and only 35.8 $\pm$ 7.8 % of particles on this coastline were fibres. This aligns with the theory that plastic pollution accumulates on the eastern shores of the Galapagos Islands as a result of exposure to the Humboldt Current transporting plastic from mainland South America to the Galapagos (Van Sebille et al., 2019; Jones et al., 2021). This long-distance transport may also account for the macroplastic accumulation zones on the eastern coast, particularly at Montones where we saw high abundances of microplastics in the seawater (10.36 $\pm$ 1.57 microplastic particles m^{-3}). Along the sheltered western coast, we identified contrasting microplastic particle morphologies; fibres were the dominant shape averaging 44.5 $\pm$ 3.4 % of particles, a maximum of 51.7 % at the Harbour, followed by fragments with a mean of 31.8 $\pm$ 8.1 % across sites. Further to the outflow pipe and proximity to urbanised areas, this dominant fibre composition co-occurs with the areas of higher boat activity along this coastline, due to the higher number of sites visited by tourists (which are strictly controlled by the Galapagos National Park) and the location of the main artisanal fishing areas. This suggests that local maritime and urban sources may also contribute to the plastic pollution along this coastline. Polymers heavily used for boat gear, e.g., polypropylene and polyethene (Nelms et al., 2021), comprised the greatest percentage of microplastic particles at sites closest to urbanisation in tow samples and subsequent intense boat activity; 61.2 % and 62.8 % at the Harbour and Carola, respectively. These spatial differences in microplastic particle morphology and polymer type within our data indicate the diverse range of plastic pollution sources in the Galapagos, with local and allochthonous sources impacting differing coastlines.

The tow data also reveal two sites with elevated polymer levels of interest; PVC was the most abundant polymer at Puerto Grande (32.5 %) and PVF (17.4 %) and plastic additives (24.9 %) were the most abundant polymers at Montones. Due to its wide applications in fishing vessels, construction and bottles, it is difficult to identify distinct sources of PVC (Chrismianto et al., 2018; Inflatex Ltd, 2023; Ur Razzaq et al., 2023); however, knowledge of localised touristic and boat maintenance activities (both artisanal fishing and tourist vessels) and the general reliance on maritime traffic to the Galapagos suggests such potential sources. Similarly, elevated abundances of PVF and plastic additives at Montones support this as either as maritime plastics applied to boats (Nakada and Miyano, 2015; Alaaeddin et al., 2019; Hu et al., 2021) or as a conspecific polymer with PVC. Both Montones and Puerto Grande are surrounded by mangroves, providing habitat for turtle species and acting as important nursery grounds for many Galapagos shark and fish species including the endangered scalloped hammerhead (*Sphyrna lewini*). The risk that PVF and PVC exposure represents to marine organisms is likely species-specific and not well studied however, polyvinyl-polymers have been shown to induce significant behavioural alterations in jellyfish (Di Giannantonio et al., 2022), and induce endocrine disruption impacting the immune system and induce cell death through ferroptosis in the fish due to the addition of di-(2-ethylhexyl) phthalate (DEHP) (Suzuki et al., 2018; McGrath et al., 2021; Chen et al., 2022) in particular (Xu et al., 2021a; Yin et al., 2021; Chiriboga-Paredes et al., 2022). These site-specific polymer abundances reveal the importance of large-scale spatial sampling to comprehend the impacts of plastic pollution at differing sites and to enable targeted and effective mitigation strategies to be implemented.

Long-term monitoring programs are imperative for guiding the Galapagos National Park in the implementation of management strategies to combat plastic pollution within the Galapagos. However, here we identify our previous understanding of plastic pollution in seawater, using traditional 200 μm tows, has vastly underestimated the abundance and size distribution of microplastics (including anthropogenic cellulose) and subsequent risk to marine life. Grab sampling can be utilised to quantify and characterise seawater microplastics accurately, informing management strategies and monitoring their success. This sampling method can be implemented with ease; citizen science has been used globally and could provide regular sample information of microplastic pollution levels and potential sources within the archipelago. This study has identified potential sources of plastic pollution in the Galapagos, including local inputs such as wastewater, emphasising the value of combining different sampling methods. Further research is needed to determine the contribution of wastewater to the Galapagos plastic problem; imperative for developing targeted local management and intervention strategies to prevent further inputs of plastic pollution, protecting the unique Galapagos marine life.

CRediT authorship contribution statement

Katie Deakin: Formal analysis, Investigation, Methodology, Writing – original draft. **Georgie Savage:** Investigation, Methodology, Formal analysis, Writing – review & editing. **Jen S. Jones:** Conceptualization, Funding acquisition, Investigation, Methodology. **Adam Porter:** Formal analysis, Investigation, Methodology, Writing – review & editing. **Juan**

Pablo Muñoz-Pérez: Investigation, Project administration, Resources. **David Santillo:** Formal analysis, Investigation, Methodology, Resources. **Ceri Lewis:** Conceptualization, Funding acquisition, Project administration, Supervision, Writing – review & editing.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Ceri Lewis reports financial support was provided by Natural Environment Research Council. If there are other authors, they 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

The research data supporting this publication are openly available from NERC EDS British Oceanographic Data Centre NOC at: https://doi.org/10.5285/0861da3d-4d76-50f0-e063-6c86abc08a7e.

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