Investigating the exposure and potential impacts of microplastics in the Galápagos marine food web

*Submitted by **Jennifer Jones** to the University of Exeter as a thesis for the degree of*

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Supervisors: Professor Tamara Galloway & Associate Professor Ceri Lewis

College of Life and Environmental Sciences, University of Exeter, Geoffrey Pope,

Stocker Road, Exeter, EX4 4QD, UK

I certify that all material in this thesis which is not my own work has been identified and that no material has previously been submitted and approved for the award of a degree by this or any other University.

Signature: ……………………………………………………………..

"Do not expect anything.

Just go with an open mind and unexpected surprises will greet you wherever you look.

But remember to look and don't hurry by...

Live the journey, for the destination may not be the expected one!

It is always worth keeping in touch with Galápagos…"

– Godfrey Merlen, Galápagos Conservation Trust Ambassador

Abstract

The Galápagos Marine Reserve is a global conservation priority, representing a vulnerable ecosystem experiencing growing anthropogenic pressures. The aim of this thesis is to investigate the sources and drivers of plastic contamination, to explore pathways and fates in coastal habitats at a variety of geographic scales (Archipelago, island and single beach) and to investigate potential impacts of microplastic contamination on marine species. Using San Cristóbal island as a case study, I collected field data (via seawater tows, benthic sediment grabs and beach survey) to test the hypotheses that (i) accumulation hotspots are present on the exposed eastern coast, (ii) beach plastic contamination is primarily from external sources to Galápagos and (iii) microplastic is present in the food web. Analysis of beach plastics > 5 mm showed a pattern consistent with the prevailing Humboldt Current as a possible driver for accumulation (> 2,500 particles m^{-2} at the most contaminated site, the east-facing Punta Pitt) with just 2% of items identified as from local sources. Evidence of microplastic uptake was observed in the digestive systems of seven marine invertebrate species including filter feeders, grazers and deposit feeders (52% of 123 individuals, mean 0.5 - 1.7 particles per individual) demonstrating entry into the food web across a range of species with different feeding modes and habitat preferences.

Using comparable environmental sampling methods, sites across two biogeographic zones in the Galápagos Marine Reserve were surveyed to test the hypothesis that contamination would be greater in the populated South-Central Zone (that has greater continental connectivity), compared to the upwelling Western Zone. The most significant differences were evident in microplastic, with concentrations in seawater, benthic sediment and sand six to ten-fold higher in the South-Central Zone suggesting a differing profile of exposure risk for wildlife across the Archipelago.

As it is not ethically nor logistically possible to sample across the entire food web, a priority scoring method was developed and applied to enable the rapid assessment of potential risks from plastic interactions for 3,159 vertebrate and invertebrate species. Data on endemism, conservation status, commercial importance (for invertebrates) and literature evidence of harmful effects were incorporated into a risk ranking system. This identified 27 vertebrate and 15 invertebrate species to be at higher risk and therefore priorities for future research and mitigation action. Finally, analysis of data collected through citizen science beach surveys showed that visual identification of suspected microplastics using a standard unit quadrat method was 93% accurate, as verified by Fourier-transform infrared spectroscopy polymer analysis (synthetic vs organic particles), providing a reliable indicator to support increased spatiotemporal resolution of beach monitoring. Overall, this work contributes to our understanding of marine plastic contamination distribution and composition in this data-poor geographic area of high conservation importance, in addition to providing several tools to support management.

Acknowledgements

I am hugely grateful to my supervisors Prof. Tamara Galloway and Ass. Prof. Ceri Lewis for their support on this journey. Without them, this PhD would not exist, let alone a multi-million pound international programme supporting the reduction of plastic waste in the Eastern Pacific that will continue this research for years to come with an inspirational team of collaborators. News crews, training with National Park rangers, two international scientific workshops, funding proposals galore and several piña coladas… it's been a busy three years! The freedom I have experienced to develop this project has been well balanced with expert guidance and positivity. A special acknowledgement must go to Ceri's wonderful fieldbooks, incorporating Darwin levels of observation skills, the backbone of our datasets and indispensable for later interpretation! I am so proud to be part of the Galloway-Lewis invertebrate ecotoxicology lab, a great community that has provided immense support with everything from complicated data analysis to pointing out the best place to get a beer in Exeter. A super special shout out has to go to my prime fieldwork buddies, Dr Adam Porter and Cathy Hobbs who have taught me so much as well as providing many laughs, also to Steph Andrews and Chloe Shute who have been fantastic writing buddies and provided much moral support.

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I dedicate this thesis to the memory of my Nan, Dorothy Jackman, who always encouraged my love of the ocean (particularly of penguins), who was so excited for me to commence this journey and without whom I would literally not be here. This one's for you!


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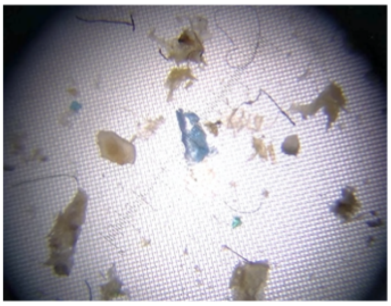





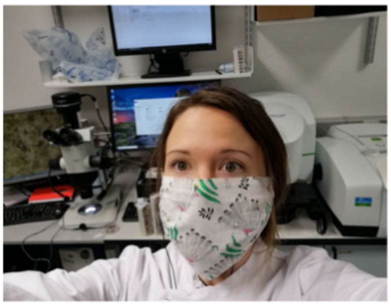
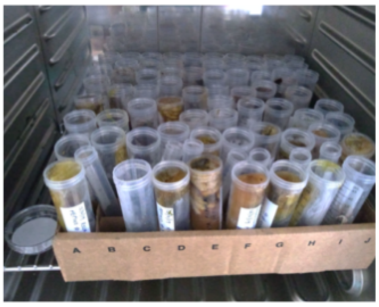







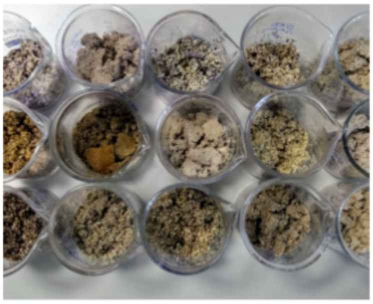







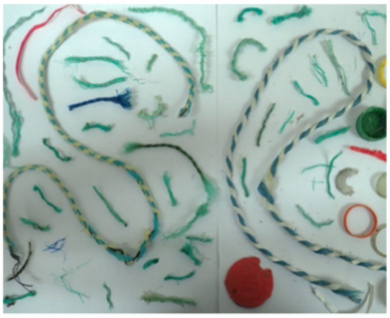


PLAYAS SIN PLASTICOS
GALAPAGOS


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Figure 6.2: Photographs of plastics on lava rubble beaches. (a) A typical lava rubble beach on western Isabela island, (b) a fragmented blue polypropylene rope, (c) attempting to collect polypropylene microfibres from a quadrat, likely from the same original green polypropylene rope (as verified by FTIR polymer analysis), (d) an example of a 'plasticrust' forming, melted into the crevices of rock and (e) an example of either side of a 'plastiglomerate' (plastic/ rock compound) that contained traces of both polyethylene and polypropylene.

Figure 6.3: Potential direct and indirect impacts from marine pollution on species adaptation amidst climate change. I designed this figure to contribute to a publication in preparation led by Juan Jose Alava: 'Multiple anthropogenic stressors reshape evolutionary processes in Galápagos: Marine pollution and climate change'.

Figure 6.4: Key pathways of marine pollution in Galápagos including oil spills (hydrocarbons), chemical pollution by persistent organic pollutants (POPs) and metals (e.g. methyl mercury, MeHg), biological pollution (e.g. invasive species and pathogens) and marine plastics. I designed this figure to contribute to a publication in preparation led by Juan Jose Alava: 'Multiple anthropogenic stressors reshape evolutionary processes in Galápagos: Marine pollution and climate change'.

Figure 6.5: Schematic of intervention points to reduce plastic waste on oceanic islands. Upstream interventions supporting a circular economy for plastics and better continental waste management to reduce the inputs (denoted in dark blue arrows) (1), at-sea interventions

supporting better waste management primarily in fisheries (2), on-island interventions e.g. improvement in wastewater treatment (3) and remediation through cost-effective, ecologically responsible clean-up (4) ideally adding value to collected plastic waste, linking back with circular economy principles in Intervention 1.

Table 6.1: Potential future research questions

Figure 6.6: "Pacific Plastics: Science to Solutions" Network. Photograph of attendees of the three-day Global Challenges Research Foundation funded workshop in Quito, Ecuador, September 2019 that launched the network with the shared goal of collaborating to reduce plastic waste in the Eastern Pacific.

Appendix 1

Figure 7.1: Photographs of particles extracted from beach sand samples. a) Blue polyethylene fragment, b) blue cellulosic fibre, c) green polypropylene soft fragment, d) black polyester fibre, e) blue polypropylene fragment, f) red nylon fibre.

Figure 7.2: Photographs of particles extracted from seawater surface samples. a) Green polypropylene fragment, b) blue polypropylene fragment, c) 12 mixed shape and polymer particles from a water tow sample in San Cristóbal harbour, d) black polyethylene fragment, e) black nylon film, f) silver polyethylene film.

Figure 7.3: Photographs of particles extracted from benthic sediment samples. a) Unidentified blue fibre, b) unidentified green fragment, c) red polyester fibre, d) blue polypropylene fragment, e) black polypropylene fibre, f) unidentified blue fragment, too small to extract.

COVID-19 Impact

I am fortunate to not have experienced the negative health impacts of the pandemic. I am indebted to my colleagues that have strived to find safe ways to continue working during what has been such an uncertain time for everyone. Like the majority of students, this project was impacted through field trip cancellations (we planned an expedition in April 2020 to sample urban wastewater, boat cleaning sites and mangrove systems, and also hoped to expand the Playas Sin Plásticos citizen science project to Isabela island) and through laboratory closures between March and December 2020 (which meant I had to remove planned heavy metals analysis due to time constraints). Spirits were kept high with the fantastically supportive culture of the lab group, however. We clubbed together to write an article on the perils of being 'Marine Biologists in Lockdown' and corralled like-minded PhD and Early Career Researchers to write an opinion piece on the future directions for microplastics research crossing our collective areas of expertise. We kept each other buoyant with talent shows, quizzes, costumes and Zoom beers, whilst supporting each other through project redesigns and extension planning.

Despite their isolation, the Galápagos Islands have been impacted by the virus like most other places, experiencing 1,646 confirmed virus cases and 20 deaths (at the time of writing in March 2021). The major economic impact of tourism shut down has been extremely distressing for the 80% of locals whose income is directly linked. The closure of the Galápagos National Park disrupted many sampling trips, but I was lucky to be involved with a remote collaboration with the Universidad San Francisco de Quito and the Galápagos National Park Directorate to survey plastics in tourist sites during COVID-19 closures. As sites are normally cleaned, sometimes on a daily basis, this represented a unique opportunity to assess plastic accumulation. Data were collected by Park Rangers and USFQ students who were trained in methods remotely by our collaborator Dr Susana Cardenas. Although it wasn't exactly the planned story, as was nothing in 2020 for anyone around the world, I am happy that this thesis achieved the overarching objectives and has proved useful in direct application in the Islands.

Author’s Declaration

My thesis is presented as six chapters (two of which have been submitted as manuscripts to peer-reviewed journals), presented here in a unified style for ease of reading. All work in this thesis has been planned, implemented and analysed by myself. Contributions of co-authors are described at the beginning of **Chapter 2** and **Chapter 5** and support for fieldwork and spectroscopy analysis is acknowledged at the end of **Chapter 4**. All references from the thesis are listed in one bibliography at the end.

List of Abbreviations & Acronyms

AMBI: AZTI Marine Biotic Index

ATR: Attenuated total reflection

°C: Degrees centigrade

cm: Centimetre

DDT: Dichlorodiphenyltrichloroethane

df: Degrees of freedom

dw: Dry weight

EEZ: Economic Exclusive Zone

ENSO: El Niño Southern Oscillation

$FeSO_4$: Iron (II) sulphate

FTIR: Fourier Transform Infrared [spectroscopy]

g: Gram

GCT: Galápagos Conservation Trust

GESAMP: Joint Group of Experts on the Scientific Aspects of Marine Environmental Protection

GNPD: Galápagos National Park Directorate

GPS: Global Positioning System

IPCC: Intergovernmental Panel on Climate Change

IUCN: International Union for Conservation of Nature

GLM: Generalised linear model

H_2O_2: Hydrogen peroxide

ind: Individual

Kg: Kilogram

Km: Kilometre

KOH: Potassium Hydroxide

L: Litre

Macroplastic: Plastic larger than 5 mm in size

MARPOL: International Convention for the Prevention of Pollution from Ships

m: Metre

μFTIR: Micro Fourier Transform Infrared [spectroscopy]

μg: Microgram

μm: Micrometre

Microplastic: Plastic smaller than 5 mm in size

mL: Millilitre

mm: Millimetre

NGO: Non-Governmental Organisation

OD: Overdispersion (re data in a GLM)

PAHs: Polycylic aromatic hydrocarbons

PCBs: Polychlorinated biphenyls

PET: Polyethylene terephthalate

POPs: Persistent Organic Pollutants

PVC: Polyvinyl chloride

QA/QC: Quality Assurance/ Quality Control

ww: Wet weight

$ZnCl_2$: Zinc chloride

Chapter 1

Galápagos and the global plastic challenge: a unique marine ecosystem facing a common problem

This introductory chapter is constructed in three parts: an introduction to the Galápagos Marine Reserve, highlighting the conservation significance of the area and history of marine pollution research (**1.1**), a global overview of marine plastic pollution with a focus on oceanic islands and the potential biological and ecological impacts relevant to the Galápagos marine food web (**1.2**), and a summary of the aims of this thesis, designed to contribute to knowledge gaps around the potential impacts of microplastics in the Galápagos marine ecosystem (**1.3**).

1.1. The Galápagos Islands, Ecuador: An introduction

Oceanic archipelagos have inspired human beings for generations, both culturally and scientifically. Although only accounting for 5.3% of Earth's terrestrial habitat, islands host 10% of the world's human population and represent unique biodiversity hotspots. Geographic isolation and vacant ecological niches promote evolutionary radiation, forging endemic species that are found nowhere else (Baldacchino, 2006; Courchamp *et al.*, 2014; Fordham & Brook, 2010). Inherently small population sizes, reduced dispersal potential and limited genetic variation may make endemic species more vulnerable to anthropogenic stressors, including habitat degradation, the overexploitation of natural capital and increasing pollution (Frankham, 1997; Hickman, 2009). This is demonstrated by the fact that 61% of known extinctions have happened on islands and 37% of critically endangered species are island endemics (Courchamp *et al.*, 2014; Tershy *et al.*, 2015). The need for significant conservation effort is clear as global extinction rates for mammals, birds and amphibians are currently 50 –

500 times higher than any historical rate in the fossil record, suggesting that we are likely witnessing Earth's sixth major extinction event with severe potential consequences for ecological functioning and services (Baillie *et al.*, 2004; Ceballos *et al.*, 2017). Furthermore, the Living Planet Report by the World Wide Fund for Nature reports a 68% decline in vertebrate species over the last several decades (WWF, 2020), a number that is likely higher due to the extinction of species that have not yet been described, particularly in marine ecosystems that are harder to sample (Costello *et al.*, 2010).

The Galápagos Islands are a volcanic archipelago situated 930 km off the coast of Ecuador in the Eastern Tropical Pacific Ocean (01°40'N - 01°25'S, 89°15'W - 92°00'W) (Fig. 1.1). There are 13 major islands and hundreds of smaller islets that are host to distinctive ecosystems, famous for their endemic biodiversity (Castro, 2005). The Archipelago is geologically young, with the western islands of Fernandina and Isabela the newest (< 700,000 years old) and most volcanically active, due to their proximity to the Galápagos hotspot (an area of super-hot magma that formed the islands). Coastal environments on these islands continue to be modified, with eruptions on both as recently as 2018 (Vasconez *et al.*, 2018). The south-eastern islands of San Cristóbal and Española are the oldest (4 – 5 million years) and have had no tectonic activity for millennia, meaning coastal habitats are more stable (Geist, 1996). Each island in the Galápagos Archipelago has ecological and evolutionary differences, with habitat diversity and zonation driven by the physical conditions and biological colonisation histories of each one (Watson *et al.*, 2010).

Since 1959, 97% of the terrestrial area of the Archipelago has been protected by the Galápagos National Park, purportedly preserving > 95% of native biodiversity and generating Galápagos' reputation as a conservation flagship area (González *et al.*, 2008). In 1986, the surrounding waters were declared a 'Marine Resources Reserve', a protection that was strengthened in 1998 as part of the 'Law of the Special Regime for the Conservation and Sustainable Development of the Province of Galápagos' which introduced the newly designated Galápagos Marine Reserve, protecting a 40 nautical mile boundary from the outer most islands (Barragan-Paladines & Chuenpagdee, 2017). Managed by the Galápagos National Park Directorate, the Galápagos Marine Reserve is multi-use (tourism, fisheries, conservation) and its management zonation is periodically reviewed. A recent iteration in 2016 saw the proposal for a large no-take zone covering 47,000 km^2 in the far north incorporating Darwin and Wolf islets, an area host to the world's largest reef fish biomass with an average of 17.5 tonnes ha^{-1}, mostly comprised of sharks (Salinas de León *et al.*, 2016). Both the Galápagos National Park and the Galápagos Marine Reserve are individually considered UNESCO World Heritage Sites due to their unique biodiversity, since 1978 and 2000 respectively (UNESCO, retrieved February 2021, https://whc.unesco.org/en/list/1). Despite the degree of protection, the Galápagos Islands were added to the World Heritage Sites in Danger List in 2007 due to the increasing risks of invasive species, unsustainable urban development and increasing tourism (Hennessy & Mccleary, 2011; Self *et al.*, 2010). It was removed from the list in 2010 as a recognition of the mitigation efforts taken by the Ecuadorian government although this decision was controversial as many consider those risks are still growing (Gross, 2010).

Socioeconomics in Galápagos

The isolation, lack of freshwater and limited agricultural potential prevented early human colonisation meaning that the timescale of anthropogenic presence is short in comparison to most other inhabited oceanic archipelagos (< 200 years), potentially increasing the vulnerability of the ecosystem to external stressors due to the lack of time to adapt (González *et al.*, 2008). Prior to the COVID-19 pandemic, it was estimated that there were approximately 30,000 people in the Galápagos Islands at any one time; approximately 4,000 tourists and transient workers and 26,000 permanent residents across the four populated islands of Santa Cruz (~16,000), San Cristóbal (~7,500), Isabela (2,500) and Floreana (150) (INEC, 2010; Toral-Granda *et al.*, 2017) (Fig. 1.1).

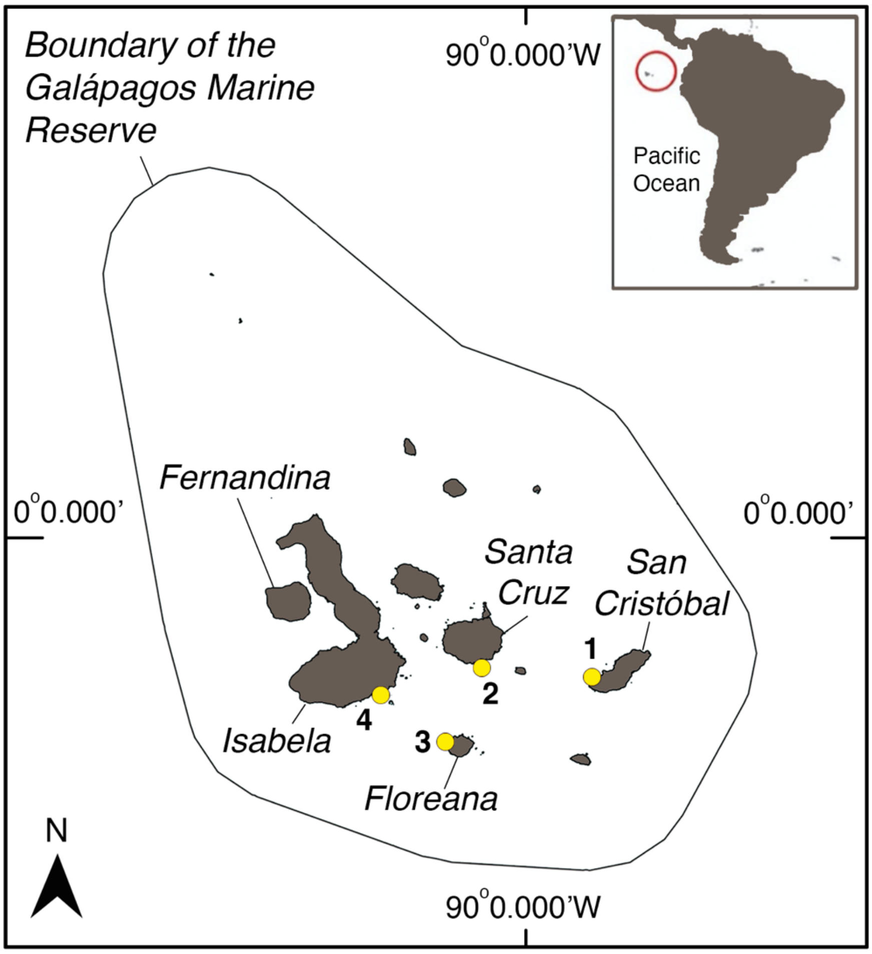

Figure 1.1: Map of the Galápagos Archipelago, Ecuador and the surrounding Galápagos Marine Reserve boundary. Location in the Pacific Ocean relative to the South American landmass indicated in the top right box by a red circle (930 km offshore). Islands featured in this thesis are labelled (Fernandina, Isabela, Floreana, Santa Cruz, San Cristóbal) and population centres are denoted by yellow circles: Puerto Baquerizo Moreno, San Cristóbal (1), Puerto Ayora, Santa Cruz (2), Puerto Velasco Ibarra, Floreana (3) and Puerto Villamil, Isabela (4).

Tourism in Galápagos has grown exponentially since the 1970s, drawing 'environmental pilgrims' from around the world. This has transformed the social situation of Galápagos, now representing > 80% of the local economy and causing a 600% increase in the resident population between 1974 and 2015 due to economic migration (Bremner & Perez, 2002;

Izurieta, 2017; Kenchington, 1989; Toral-Granda *et al*., 2008). Migration to Galápagos is now strongly controlled although there is no cap on tourist numbers; in 2019, 271,000 tourists visited representing a 660% increase since 1990 (41,000 tourists) (Epler, 2007; GNPD, 2019; Izurieta, 2017). This increased traffic of people and cargo provides new channels for species introductions, compromising the biological isolation of the Islands. A recent study by Toral-Granda *et al.* (2017) described 1,476 invasive species that have already become established including five marine invertebrates and two species of marine macroalgae that likely arrived in boat ballast water (Keith, 2016; Toral-Granda *et al.*, 2017). Increasing marine traffic also carries the risk of increased oil spills and other environmental contamination such as from persistent organic pollutants (POPs) and anti-fouling paints (Alava *et al.*, 2014).

After tourism, the artisanal fishing fleet is the second largest employer in Galápagos. Since industrial fishing in the Galápagos Marine Reserve was banned in 1998, the size of the artisanal fishing fleet has remained relatively stable with approximately 1,000 fishers and 450 boats registered (Bucaram & Hearn, 2014; Castro, 2005). An analysis of Ecuadorian fishery landings between 1950 and 2010 by Schiller *et al.*, showed a total of 797,000 tonnes caught in the Exclusive Economic Zone of Galápagos, mostly comprising industrially caught tuna fished outside of the Galápagos Marine Reserve (80%) and a considerable proportion of sharks (13%, 103,610 tonnes), mostly illegally caught for the fin trade (Schiller *et al.*, 2014). Within the artisanal fishery inside the Galápagos Marine Reserve in the same time period, a total of 26,500 tonnes of finfish was caught (25% locally consumed, 75% exported). Marine invertebrates represent an important part of the artisanal fishery (explored further in **Chapter 3**). In this same 60 year period, the seasonal fishery caught 9,200 tonnes of spiny lobsters (*Panulirus penicillatus* and *Panulirus gracilis*) (7% locally consumed, 93% exported primarily to the United States of America, valued at $1,000,000 in 2012) and 800 tonnes of slipper lobster (*Scyllarides astori*) (88% locally consumed) (Ramirez *et al.*, 2013; Schiller *et al.*, 2014). Following the collapse of Ecuadorian mainland sea cucumber populations, an open-access fishery was started in Galápagos, resulting in 16,100 tonnes of brown sea cucumber

(*Isostichopus fuscus*) caught within the EEZ in this time period (19% illegally), all of which were exported for the Asian market (Schiller *et al.*, 2014).

The trade-off between the conservation of the natural environment and the development needs of the resident human population are a continual balancing act; commonly cited as the "Galápagos paradox" due to the pristine nature of the system being the main driver for economic income (via tourism and fisheries), at the same time as the tourism and fisheries industries presenting some of the biggest threats to the ecologically integrity of the area (Izurieta *et al.*, 2018; Pizzitutti *et al.*, 2017; Walsh & Mena, 2016).

Oceanography and climate

Both the tourism and fishing industries, and ultimately the entire biodiversity of the Archipelago, are supported by the high productivity of the Galápagos marine ecosystem. This differs markedly from the generally high nutrient, low chlorophyll profile of the rest of the equatorial Pacific due to the interactions of several oceanographic and climatic processes (Fig. 1.2). The Intertropical Convergence Zone (ITCZ) is a circum-global low-pressure zone where the trade winds of the northern and southern hemispheres meet, forming a boundary in the Eastern Pacific between the warm, oligotrophic waters of the Panama Current to the north and the cooler, more productive waters from the Humboldt Current to the south (Palacios, 2004). The relative position of the ITCZ drives the two seasons in Galápagos; the warm-wet season (December to May) when the Panama Current is prevalent and the cool-dry 'garúa' season (June to November) when the Humboldt Current dominates (Edgar *et al.*, 2004a; Palacios, 2004).

As the waters from the Panama and Humboldt Currents mix and flow to the west, they form the South Equatorial Current. The westward flow of the South Equatorial Current causes the cool, sub-surface Equatorial Undercurrent to flow in the opposite direction along the equator, back towards Galápagos. The steep shelf to the west of the Galápagos platform (the relatively shallow < 200 m platform that connects the central islands (Geist *et al.*, 2008)), causes a strong topographic upwelling of the Equatorial Undercurrent strengthened by the additional effect of northward winds driving localised sub-mesoscale flows (< 10 km) that have only very recently been described (Forryan *et al.*, 2021). Upwelling cools sea surface temperatures and acts as an important nutrient input. This is in the form of nitrates that, along with the iron derived from the volcanic platform itself, are the major nutrient drivers for marine primary production and thus the foundation of the entire marine food web (Palacios, 2004; Schaeffer *et al.*, 2008). Although the effects of the Equatorial Undercurrent upwelling are strongest in the west, branches travel throughout the marine reserve resulting in several areas of high productivity throughout the central islands (Schaeffer *et al.*, 2008).

Seasonal variation in sea surface temperature around Galápagos is significant, particularly during El Niño Southern Oscillation (ENSO) events (Schaeffer *et al.*, 2008). ENSO is a Pacific climatic phenomenon borne from the oscillation of warm (El Niño) and cool (La Niña) states and is considered the strongest interannual climate fluctuation on Earth (Timmermann *et al.*, 1999). It is measured by the Oceanic Niño Index; the running three month mean sea surface temperature anomaly for the ENSO region (5°N – 5°S; 120°W – 170°W). An ENSO event occurs when the Oceanic Niño Index has an anomaly of ± 0.5°C for five consecutive months, tending to last about 18 months and recurring every 3 – 7 years (Atwood & Sachs, 2014). Ecological impacts are pronounced due to the interplay between abiotic factors, e.g. warming temperatures, changing wave action and sea level change, and biotic factors, e.g. bottom up forcing of changing primary production and algal community composition and top down forcing from changes in grazing pressure, causing high dynamism particularly in intertidal ecosystems (Schaeffer *et al.*, 2008; Vinueza *et al.*, 2006; Wingfield *et al.*, 2018).

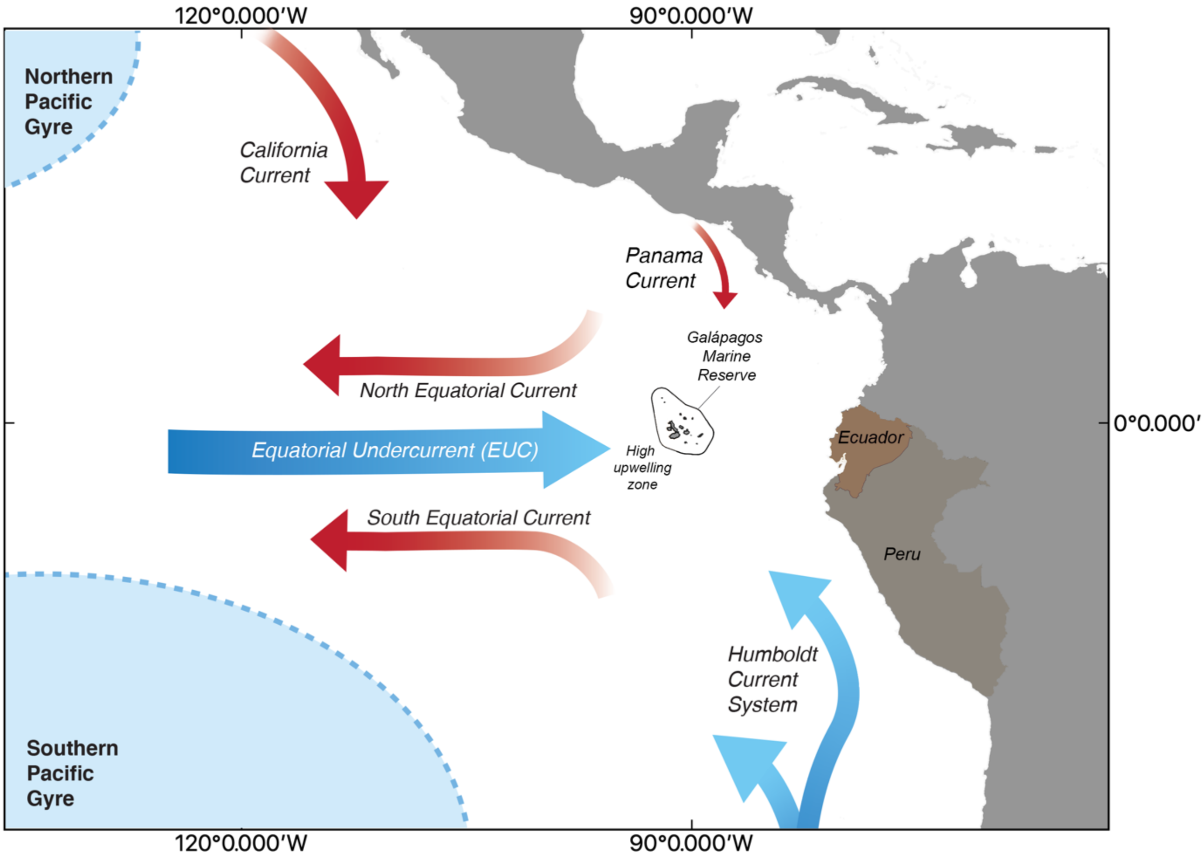

Figure 1.2: Map showing the major current systems and oceanographic features surrounding the Galápagos Marine Reserve. Cold-water currents are denoted by blue arrows and warm-water currents by red arrows.

Characterising coastal ecosystems in the Galápagos Islands

The total length of coastline of the Galápagos Islands is approximately 1,800 km, characterised by exposed lava rocky shores interspersed with mangroves (estimated to cover 35%, see fcdgps.maps.arcgis.com) and small beaches with sediments ranging from echinoderm tests and coral rubble to eroded lava (Alava *et al.*, 2014; Castro, 2005). Coastal environments are the interface between terrestrial and marine ecosystems, and provide many essential ecosystem services including sediment storage, water filtration, nutrient cycling (Defeo *et al.*, 2009) and provision of important habitats for many threatened endemic species including the marine iguana (*Amblyrhynchus cristatus*), the Galápagos sea lion (*Zalophus wollebaeki*) and seabird colonies including the flightless cormorant (*Phalacrocorax harrisi*) and

Galápagos penguin (*Spheniscus mendiculus*). This megafauna is supported by a diverse algal and invertebrate community at the base of the food web that varies by biogeographic zone (see **Chapter 4**).

To become established in this highly variable environment, species have adapted to cope with significant environmental variation over relatively short spatial and temporal scales imposing a strong selective pressure for species that have high tolerance ranges and/or the ability to bounce back quickly after severe population declines (Glynn *et al.*, 2018). This selection is likely a major driver for the relatively high percentage of endemic marine species within the Galápagos Marine Reserve, estimated at 18.2%, qualifying Galápagos as the only tropical archipelago with sufficient marine endemism to be considered a biogeographic province in its own right in a global review (Briggs & Bowen, 2012).

Marine contamination in Galápagos

Galápagos represents an interesting system to investigate the responses of naïve, endemic species to challenges from anthropogenic pollution. Currently it is not known whether endemic species will be more sensitive to chemical threats or be more tolerant due to the inherent stress of the high environmental variation and the natural exposure to potential chemical stress from the heavy metals derived from the volcanic geology of the Islands (Franco-Fuentes *et al.*, 2021; Jiménez *et al.*, 2020). The differential responses to pollution between native and non-native species have been tested in other geographic areas with mixed results. In Australia, Piola & Johnston (2009) showed increased tolerance to metal pollution (copper) in cosmopolitan, non-native bryozoans compared to indigenous species, measured by growth and recruitment rates and recovery following exposure (Piola & Johnston, 2009). In contrast with the paradigm that non-native species are more tolerant to chemical stress however, Ferrario *et al.* (2020) report increased tolerance of native fouling communities in Madeira island (North-East Atlantic) to zinc oxide anti-fouling paints compared to communities of non-native species (Ferrario *et al.*, 2020). Delineating the potential evolutionary responses to increasing anthropogenic pollution and climate change scenarios will be increasingly important in Galápagos to reduce risk of invasive species establishment and evolutionary changes as a result of the multi-stressor impacts of the Anthropocene (Boyd *et al.*, 2018).

Due to the small human population and strong environmental protection, the Galápagos Marine Reserve is assumed to have low local pollutant inputs although long-range atmospheric transport is possible for some persistent organic pollutants (POPs) (Alava & Ross, 2018). Nonetheless, there are several local contamination sources of concern including from agriculture (especially pesticides), sewage and wastewater run-off, maritime activity (oil spills, hydrocarbon emissions and ballast water) and solid waste including the incineration of plastics and organic waste presenting a potential source of dioxins and furans (Alava *et al.*, 2014). Historical usage of the insecticide Dichlorodiphenyltrichloroethane (DDT) in the 1940s at the American airbase on Baltra island appears to still be traceable in Galápagos sea lion (*Zalophus wollebaeki*) populations, with 8 – 9% of surveyed sea lions exceeding the p,p'-DDE immunotoxicity threshold which can lead to reproductive impairment and carcinoma in other sea lion species (Alava-Saltos, 2011). Although chemical pollution is not frequently acknowledged as one of the biggest threats to the Galápagos marine ecosystem, there is currently no robust baseline for many POPs including heavy metals and polychlorinated biphenyls (PCBs) meaning that currently, the risk profile is unknown (Izurieta *et al.*, 2018).

Before I started this thesis in April 2018, no studies describing marine plastic pollution in Ecuador and Galápagos had been published, despite rising concerns in NGO networks and the Galápagos National Park Directorate that over the last twenty years, the amount of plastic accumulating in the Galápagos Archipelago was increasing considerably, primarily assumed to be from external sources. In the following section, I introduce the global context of marine plastic pollution and summarise the current status of research.

1.2. Marine plastic pollution: Distribution, sources and impacts

Marine plastic pollution: A global crisis?

Plastic contamination is certainly ubiquitous in the natural environment; found in all five oceanic gyres (Eriksen *et al.*, 2014), in Arctic sea ice cores (Obbard *et al.*, 2014), at more than 10,000 m depth in the Marianas Trench (Chiba *et al.*, 2018), at the top of Mount Everest (Napper *et al.*, 2020) and even in the air (Dris *et al.*, 2017). It is estimated that the natural capital cost of plastic pollution to the global marine environment is $13 billion (USD) when considering degraded habitats, revenue loss for fisheries and tourism and the cost of clean-up (UNEP, 2014). The ultimate fate of most mismanaged plastics is the ocean, and as some ecological impacts are probably irreversible, plastic pollution has been proposed as severe enough to be considered a Planetary Boundary threat (Galloway & Lewis, 2016; Villarrubia-Gómez *et al.*, 2018). Plastics have also been proposed as POPs according to the definition from the Stockholm Convention on POPs as 'potentially harmful organic compounds that resist environmental degradation through chemical, biological and photolytic processes' (Galloway *et al.*, 2017; Worm *et al.*, 2017).

There is some debate around the amount of focus on plastic pollution in the context of other environmental challenges such as climate change or food security, however, bringing into question whether inflammatory vocabulary such as it being a 'global crisis' is truly warranted. Concerns include the introduction of weak policies by Governments to tackle plastic pollution without evidence of the effectiveness of said interventions (not dissimilar to corporate 'green-washing') and also the often un-tested assumption that plastic action might be a 'gateway' to more sustainable behaviours from consumers in other areas (Stafford & Jones, 2019a, 2019b). Regardless, there is a global consensus that plastic pollution is a growing problem and a strategic, interdisciplinary approach is necessary to 'turn off the plastic tap' to avoid further ecological and socioeconomic impacts (Ellen MacArthur Foundation, 2016). There is also an argument that plastic pollution is inherently linked with other global challenges, including climate change (explored further in **Chapter 6**), suggesting that well-targeted action to reduce plastic waste will likely have positive impacts on reducing other threats (Pahl *et al.*, 2017; Zhu, 2021).

How much plastic is in the marine environment?

The first step to assess the risks of a novel pollutant, is often to quantify environmental concentrations (Nordberg *et al.*, 2009). In the marine environment, sampling efforts have historically been focused at the seawater surface due to the ease of sampling. In an attempt to estimate the amount of floating sea surface plastic in the global ocean, Erikson *et al.* developed a model based on data from 24 cruises across all five sub-tropical gyres resulting in an estimate of 5.25 trillion plastic particles (258,940 tonnes) (Eriksen *et al.*, 2014). This was corroborated by another study by Van Sebille *et al.*, estimating 15 – 51 trillion floating plastic particles with a comparable weight range of 33,000 – 236,000 tonnes (Van Sebille, *et al.*, 2015). Although plastic inputs are lower in the southern hemisphere, oceanographic models estimate a similar floating plastic load to the northern hemisphere suggesting that plastics can traverse entire oceans (Eriksen *et al.*, 2014). Sampling effort is not evenly distributed, focused on the main accumulation zones of the sub-tropical gyres and highly populated coastal areas suggesting the potential for errors in global datasets until geographical data gaps are addressed (Eriksen *et al.*, 2014). Furthermore, the majority of sea surface sampling has used plankton nets with 333 μm mesh, suggesting that these quantities are under-estimates, missing the smallest size fractions of plastic and fibres that are thin enough to pass through the nets (Brandon *et al.*, 2020). It is also important to note that floating plastic only makes up a very small contingent of global marine plastic pollution (estimated < 1%) with the majority expected to sink to the benthos (Van Sebille *et al.*, 2015; Kaiser *et al.*, 2017; Porter *et al.*, 2018). Plastic studies in ecologically sensitive Marine Protected Areas are scarce in the literature. Due to higher biodiversity and biomass, organisms are potentially more likely to encounter plastics, even if contamination is lower overall. It is therefore possible that biological and ecological risk may not necessarily be highest in areas of highest contamination meaning that sampling in important conservation areas such as the Galápagos Marine Reserve should be a priority.

What are plastics and why do we use so much?

To understand why plastics are so persistent in the environment, we need to consider their chemical composition and how they are manufactured. Plastics are anthropogenically manufactured polymers comprised of repeating units (monomers, Fig. 1.3), often made from fossil fuel or bio-based hydrocarbons with additives such as plasticizers, stabilizers and flame retardants. They can be either thermoplastics (can be melted and reshaped) or thermoset plastics (cannot be melted and reformed) with their physicochemical properties defining their attributes for different applications (PlasticsEurope, 2018). Since the 1940s, plastics have provided a cheap, light, durable and generally inert material to manufacture a variety of products that are now part of our everyday lives bringing many notable societal benefits supporting medical advances, improving health and safety, reducing food waste and saving energy (Andrady & Neal, 2009). If products are designed with end-of-life in mind as per the principles of the circular economy (Ellen MacArthur Foundation, 2016), some plastic polymers can be reused and recycled extensively, representing a lower environmental footprint than many alternative materials. Unfortunately however, much of the virgin plastic produced is used for almost valueless single-use packaging; comprising an estimated 40% of all production in Europe (PlasticsEurope, 2018). After a short usage period, sometimes in the order of seconds, the disposal of single-use materials represents an estimated $80 billion lost to the economy and it is predicted that 32% of this material ends up leaked into the environment (Ellen MacArthur Foundation, 2016).

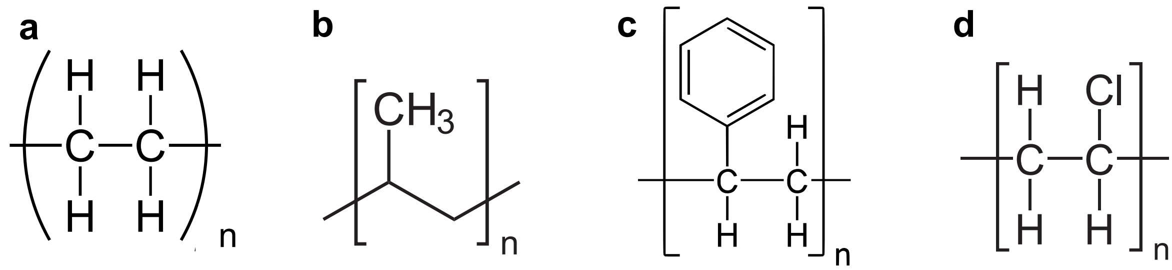

Figure 1.3: Monomers of commonly produced plastics. (a) Polyethylene (29.7% of plastics demand by resin type in the European Union in 2018), (b) polypropylene (19.3%), (c) polystyrene (6.4%), (d) polyvinyl chloride (PVC) (10%) (data from PlasticsEurope, 2019).

The global rate of plastic production has grown from 1.7 million tonnes in the 1950s to 322 million tonnes in 2015 although in Europe, the production rate has been relatively stable for the last ten years (Worm *et al.*, 2017). Up to 2015, it is estimated that the total amount of virgin plastics produced was 8,300 million tonnes, and apart from waste that has been incinerated (12%), the remainder still exists, either recycled into new products (9%) or more usually accumulated in landfill or the natural environment (79%) (Geyer *et al.*, 2017). The properties that make plastics so useful, present a major challenge for their disposal as they do not biodegrade, instead persisting on long timescales in the environment, fragmenting into smaller and smaller pieces (Andrady & Neal, 2009; Geyer *et al.*, 2017). Table 1.1 describes common polymers extracted from environmental samples with their density noted (g cm^{-3}).

Table 1.1: Common polymers in order of low to high density, commonly extracted from marine environmental samples (seawater and sediment) (after Andrady, 2011; PlasticsEurope, 2018; rsc.li/progressive-plastics-report).

Polymer Type	Common Uses	Density (g cm^{-3})
Expanded Polystyrene	Packaging especially in fisheries	0.01 – 1.05
Polypropylene	Rope, containers, automotive parts, toys	0.90 – 0.92
Polyethylene	Packaging films, carrier bags, food containers (low density polyethylene), chemical containers, industrial packaging (high density)	0.91 – 0.95
Density of seawater 1.02 g cm^{-3}		
Polystyrene	Food packaging, medical consumables	1.04 – 1.09
Polyamide (nylon)	Fishing lines, clothes	1.13 – 1.15
Polyvinyl Chloride (PVC) aka poly(chloroethane)	Pipes, wiring, sheets	1.16 – 1.40
Cellulose Acetate	Cigarette butts, sanitary products	1.22 – 1.24
Polyethylene Terephthalate (PET) aka polyester	Drinks bottles, insulation, textiles (often blended with natural fibres e.g. cotton)	1.34 – 1.39
Density of zinc chloride (used in sediment:microplastic flotation protocol) 1.5 g cm^{-3}		

Microplastics

The term “microplastic” was first coined in 2004 when researchers modelled that particles < 5 mm likely made up the major fraction of plastic ‘missing’ in environmental samples (Thompson *et al.*, 2004). Research progress was initially slow, but recently the publication rate has grown exponentially as the field rapidly expands; increasing from 19 studies in 2009 to 1,023 in 2019 (Qin *et al.*, 2020). A lack of consistency in sampling approaches and data reporting is a commonly cited barrier to inter-study comparisons to progress our global understanding of the issue (reviewed by Burns and Boxall, 2018), although several attempts have been made by organisations such as the Group of Experts on the Scientific Aspects of Marine Environmental Protection (GESAMP) to harmonise approaches. The definition of what particle size constitutes a microplastic differs between researchers. A threshold of 1 μm - 5 mm is most commonly used, to include pre-production pellets (aka nurdles) that are often 3.5 – 4.5 mm in diameter (GESAMP, 2015). More recently, a shift to referring to microplastics as particles < 1 mm has been proposed in an effort to standardise reporting, fitting more traditionally with metric measurements (Frias & Nash, 2019; Hartmann *et al.*, 2019). In this thesis, I adopt the traditional size range, considering large microplastics as 1 – 5 mm and small microplastics as 1 μm - 1 mm.

Depending on their origin, microplastics may be defined as primary or secondary. Most plastic products start as pre-production pellets which are considered primary microplastics. Other primary microplastics include microbeads used for abrasive cleaning, from boats to human faces (UNEP, 2016). Environmental contamination by primary microplastics was first recorded in 1971 in the Sargasso Sea, and the possibility of association with toxic compounds was postulated immediately (Carpenter *et al.*, 1972). Although environmental leakage clearly occurs, primary microplastics are now less frequently found in the environment than secondary microplastics, particles that are created from the fragmentation of larger plastics or via introduction from sewage or wastewater e.g. fibres from clothing (Browne *et al.*, 2011; Burns & Boxall, 2018; Weinstein *et al.*, 2016).

Plastics in the environment are subject to variable weathering and fragmentation processes that cause the generation of increasingly smaller particles. Exposure to sunlight and ultraviolet (UV) radiation in areas of high oxygen availability can alter the chemical structure of plastic polymers through photo- and thermo-oxidative processes causing bond cleavage and a reduction in structural integrity (Andrady, 2011). Fragmentation is accelerated with mechanical stress from wave and wind action and is much faster in the beach environment where oxygen availability is higher than in seawater (Andrady, 2011). Due to the equatorial position of the Galápagos Islands, a high UV Index is likely to speed up the fragmentation of plastic beach litter. Measurements from cities in mainland Ecuador show UV levels often exceeding the threshold to be considered ‘extreme’ by the World Health Organisation, increasing with elevation in the Andes but nevertheless high along the coast which is likely to be in the same region as levels in Galápagos (Harari Arjona *et al.*, 2016).

In addition to the physical and chemical weathering mentioned above, biological interactions also modulate the production, movement and fate of microplastics in the environment. Marine vegetation has been shown to impact microplastic retention in sediments, seen in mangrove systems (Naji *et al.*, 2019), seagrass meadows (Plee & Pomory, 2020) and macroalgal canopies (Cozzolino *et al.*, 2020). Furthermore, microplastics can accumulate on the surface of plant matter, posing a potential ingestion pathway for marine herbivores. Biota also play a role in fragmentation, for example, via the mechanical actions of grazers as demonstrated in the laboratory with the sea urchin (*Paracentrotus lividus*) by Porter *et al.*. Individuals generated > 90 microplastic particles each over 10 days grazing on bio-fouled macroplastic crates (Porter *et al.*, 2019). Some organisms inhabit the plastic itself if soft enough, such as burrowing polychaete worms living inside floating expanded polystyrene buoy debris, able to produce > 1,600 microplastics per individual in a single day (Jang *et al.*, 2018). Upon ingestion, microplastics may also be significantly modified by physical and chemical processes involved with digestion that may work to breakdown polymers or even aggregate them (Watts *et al.*, 2014).

Biofouling by invertebrates and algae can alter the density of particles meaning that low density polymers such as polypropylene and polyethylene that would normally float, can sink through the water column, accumulating in sediments (Fazey & Ryan, 2016). Incorporation in marine snow has been demonstrated as a potential faciliatory mechanism for the downward flux of microplastics to the benthos (Porter *et al.*, 2018). The formation of biofilms also affects palatability influencing the likelihood of uptake into food webs with composition shown to vary with polymer type and salinity (Ramsperger *et al.*, 2020). Ingested microplastics may accumulate in organisms but are also egested in faeces facilitating transport to the benthos as seen in copepod faecal pellets (Cole *et al.*, 2016). Once in the benthic environment, microplastics may be buried and resuspended by geophysical processes (Zhang, 2017) or by the actions of bioturbators such as burrowing polychaetes and brittlestars that have been hypothesised to potentially sequester microplastics for millenia (Coppock *et al.*, 2021).

Biological and ecological impacts of marine plastic pollution

Recognised adverse impacts from biotic interactions with plastics span the subcellular to the ecosystem scale and the difficulty of observing and measuring effects varies by taxa (Galloway *et al.*, 2017). Measurable biological endpoints vary from temporary enzyme disruption to mortality (Table 1.2). Research on the negative interactions between wildlife and plastics began in the 1960s, focused primarily on the very visible issue of entanglement of seabirds and pinnipeds (Ryan, 2015a). Effects from entanglement vary in severity from laceration to limb loss, restricting movement and potentially resulting in starvation or disease (Vegter *et al.*, 2014). Entanglement may also result in respiration effects due to the impairment of gills as reported in blue sharks (*Prionace glauca*) where gills were damaged by plastic packaging bands (Colmenero *et al.*, 2017) or in a deceased whale shark (*Rhincodon typus*) in Indonesia, where a single-use drinking cup was observed lodged in the gills (Abreo *et al.*, 2019).

Deleterious effects from the ingestion of macroplastic has been described in many marine vertebrates, including in the South Eastern Pacific, linked with injury and increased mortality in seabirds, sea turtles, fish, pinnipeds and cetaceans (Thiel *et al.*, 2018). Determining fitness consequences linked with sub-lethal plastic ingestion in vertebrates is difficult however, as producing dose-response relationships is not possible due to ethical reasons (Wilcox *et al.*, 2018). Recent ecological modelling advances have addressed some of these knowledge gaps for higher vertebrates, although they depend on existing knowledge of physiology, life history traits and encounter rate (Compa *et al.*, 2019). Nonetheless, models are becoming increasingly sophisticated and are growing in scale from a taxa focus (Schuyler *et al.*, 2016;

Wilcox *et al.*, 2015) to whole ocean basin ecosystems, demonstrating the value also of working across biological and oceanographic disciplines (Compa *et al.*, 2019).

Table 1.2: Recognised adverse impacts of microplastics exposure from ingestion including example effects at subcellular, cellular, organism and population level (after Galloway & Lewis, 2016).

Subcellular	Cellular	Organism	Population
• Oxidative damage • DNA damage	• Elevated antioxidant response • Apoptosis • Altered fatty acid metabolism	• Behavioural disruption e.g. feeding • Increased metabolic demand/ energy budget • Depletion of energy reserves • Respiration effects • Immune response • Mechanical injury e.g. from sharp fragments • Oxidative stress response	• Reduced growth • Decreased reproductive success • Decreased offspring viability • Increased mortality

The likelihood of ingestion of microplastics by marine invertebrates has traditionally been related to their ability to ingest particles of a certain size range with the prevailing assumption that microplastics are increasingly bioavailable the smaller they are, although density, abundance, colour and biofouling are all likely factors that influence encounter rate (Wright *et al.*, 2013). Ingestion by invertebrates was first demonstrated in laboratory exposure studies (e.g. Browne *et al.*, 2008; Cole *et al.*, 2013; Von Moos *et al.*, 2012) but is now supported by a wealth of field-based data showing microplastic contamination in at least 329 wild species (Miller *et al.*, 2020), although this is likely a vast underestimate when considering the increasing frequency of new reports.

There is now good evidence across a number of different species that the retention of microplastics can cause satiation, often reducing feeding activity due to physical obstruction, or by disrupting digestive enzymes, the absorption of nutrients and the storage of fat reserves, compromising fitness (Brennecke *et al.*, 2015; Derraik, 2002). Micro- and nanoplastic exposure studies with molluscs, crustaceans and annelids using environmentally relevant concentrations demonstrate dose-dependent, chronic sub-lethal health effects such as reduced digestion and disruption of embryonic development (Galloway *et al.*, 2017; Haegerbaeumer *et al.*, 2019). These impacts are likely species-specific due to differences in metabolic rates, diets, morphology and feeding behaviour and thus are challenging to quantify (Donnelly-Greenan *et al.*, 2014). Equally, there are likely to be species-specific effects in egestion ability and therefore likelihood of retention (Ory *et al.*, 2018).

These physiological responses also affect the potential utility of species as 'bioindicators' to monitor environmental microplastic contamination. Bioindicators are often an important part of ecotoxicological data collection to inform policy-makers of the potential wider ecological effects. Embracing the 'canary in the coalmine' theory, good bioindicators are moderately tolerant to stressors and are not too rare, ideally showing sub-lethal responses that are easy to measure (Holt & Miller, 2010). A variety of species have been recommended as bioindicators for plastic contamination, from static macroalgae (Feng *et al.*, 2020), to invertebrates such as jellyfish, bivalves and barnacles (Li *et al.*, 2019; Macali & Bergami, 2020; Xu *et al.*, 2020), to higher vertebrates such as seabirds and cetaceans (R. C. Moore et al., 2020; Phillips & Waluda, 2020).

The lack of information around the chemistry of plastics, their environmental movement pathways and their fate upon contact with an organism makes it difficult to measure physiological responses to model associated ecological impacts and establish acceptable environmental risks (Vegter *et al.*, 2014). The definition of 'risk' can vary significantly between scientific disciplines. In ecotoxicology, it is generally considered that 'risk = exposure x hazard' requiring data on environmental concentrations of pollutants, encounter rates and negative health effects on species in order to establish acceptable environmental thresholds. The merits of adopting a more precautionary approach whilst the research community is compiling further evidence is debated in an article (that originated as a Twitter debate) between Backhaus and Wagner (2020). The persistence of microplastic and our current inability to measure long-term effects of exposure for organisms suggests that it may not be wise to wait for the evidence before interventions are started to reduce microplastic leakages into the environment (Backhaus & Wagner, 2020). From an ecological standpoint, degree of risk might also consider effects on ecosystem services, with keystone species, commercially important species and particularly range-restricted populations of high concern (Zacharias & Roff, 2001).

Mitigating adverse effects of plastic contamination

Plastic pollution captures people's attention; from the highly emotive imagery of injured charismatic wildlife, concern about human health impacts and the relatable experience of polluted coastlines affecting perceptions of a place and subsequent well-being (Pahl *et al.*, 2017; Wright & Kelly, 2017). Perhaps this interest is also fuelled by the collective guilt that we are all complicit through our societal over-consumerism, therefore we are simultaneously motivated to engage in solutions, **as long as they are easy**. The translation of this interest into behaviour change has not yet been measurable, exhibited by the infamous '*Blue Planet II*' effect, where although there was a positive influence on awareness, this has not translated to active change in viewers' consumer choices (Dunn *et al.*, 2020). Although people clearly care about this issue, a pluralistic approach engaging policy makers, industry and consumers is necessary and robust baseline environmental and ecological data is critical to design and measure the effectiveness of appropriate interventions.

1.3. Context of this Thesis

At a global scale, Latin America and particularly the Eastern Pacific region, is poorly sampled for plastic contamination, including on beaches (Serra-Gonçalves *et al.*, 2019), at the seawater surface (Van Sebille *et al.*, 2015) and for negative wildlife interactions (Nelms *et al.*, 2016). There is a major bias to the 'westernised' Northern Hemisphere in microplastics research with the top five countries in terms of output being the United Kingdom, United States of America, Germany, France and the Netherlands, unlikely to represent areas experiencing the most severe impacts of plastic pollution whether from an ecological or a socioeconomic perspective (Qin *et al.*, 2020). Ecuador has one of the lowest publication counts for plastic pollution in Latin America, particularly for microplastics (n = 2 in 2020; Kutralam-Muniasamy *et al.*, 2020). This is postulated to be due to economic and capacity constraints, with the lack of government investment in scientific careers and absence of national PhD programmes contributing to Ecuador being considered as 'scientifically lagging' (Kutralam-Muniasamy *et al.*, 2020). Despite these challenges, research has begun, with the identification of microplastics in drinking water and manufactured drinks, and the contamination by tyre particles in the city of Riobamba (Paredes *et al.*, 2019, 2020). No publications describing marine microplastics in Ecuador were found at the time of writing however, demonstrating a major knowledge gap.

Beach surveys for larger plastics have only started very recently in Ecuador with the first national sampling of beach plastics (focusing on macroplastic > 25 mm) undertaken by a volunteer network in 2019, identifying tourist inputs as the primary source of beach litter on the mainland (Gaibor *et al.*, 2020). One Galápagos beach was included in the national survey (Tortuga Bay, Santa Cruz island, one of our study sites in **Chapter 5**). This site was considered extremely clean compared to the mainland sites which is perhaps unsurprising as it is cleaned almost daily, preventing macroplastic accumulation. This concurs with Mestanza *et al.* who also reported that macroplastic accumulation on Galápagos tourist beaches (n = 8) was much lower than in the mainland; attributed to small human population, no riverine input, elevated environmental expectations of visitors and good provision of bins and awareness messaging (Mestanza *et al.*, 2019). This study again focused on accessible tourist sandy beaches that are regularly cleaned and located mostly in sheltered bays, not representing the variability of plastic accumulation on more remote, unvisited, exposed coastlines subject to floating debris brought by oceanic currents. The quantification and description of contamination in these habitats is essential to understand the true plastics budget of the Galápagos Archipelago to consider impacts across the whole marine food web and to delineate sources. These findings, in addition to anecdotal evidence, contribute to the hypothesis that unlike the mainland, the primary source for plastic contamination in Galápagos is unlikely to be from tourism.

That is not to say that there isn't considerable local generation of plastic waste, but as yet, no data are available to calculate potential leakage. A report published by WWF and Toyota in 2010 estimated that 20 tonnes of domestic waste was produced daily in Galápagos with 16.8 tonnes generated from households and commercial businesses and 3.2 tonnes per day from tourism boats. An average 3% of household waste was plastics, equating to 532 kg produced each day. The authors predict that waste produced on the Islands doubles every ten years – not only attributed to population growth but also to new and increased consumption habits (Torsten Hardter *et al.*, 2010). If this prediction is correct, this would suggest that in 2020, over a tonne of plastic waste was produced in Galápagos on a daily basis.

In Galápagos, some of the most significant knowledge gaps concern: i) the identification of sources and sinks of plastics contamination, highlighting hotspots, ii) the elucidation of plastic pathways in the environment, and iii) the potential biological and ecological impacts this contamination may cause. Without establishing a baseline knowledge in these three areas, we cannot know how concerned we should be or how to most efficiently tackle the problem, as suggested for other systems (Eriksen *et al.*, 2014; Mendenhall, 2018). Anecdotal evidence from local researchers and practitioners of increasing marine plastic contamination catalysed the formation of the 'Plastic Pollution Free Galápagos' Programme in 2018; a multi-institutional initiative designed to support the Galápagos National Park Directorate to tackle this issue (Galápagos Conservation Trust, 2018; https://tinyurl.com/tfdc9yv, last retrieved February 2021). The collective aim of this multi-disciplinary, international alliance was to fill the major knowledge gaps around marine plastics and the Galápagos system as well as to address capacity issues to ensure the translation of research findings into management action. So far, the network has published modelling approaches using virtual floating plastics transported on ocean surface currents that have identified continental inputs as a major source of plastic input to Galápagos, 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; a publication where I was a co-author). Models suggest that only a small amount of plastic is entering Galápagos 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; a second publication where I was a co-author). The lack of field data was identified as a major barrier to our understanding of plastics in the Galápagos Marine Reserve.

Summary of thesis aim and objectives

Aim: To develop an understanding of the composition and distribution of plastic contamination in the Galápagos Marine Reserve and to investigate potential impacts of microplastic contamination on marine species.

Specifically, the objectives were:

(i) To describe marine plastic contamination at an island scale, identifying potential risks to native marine vertebrate species (**Chapter 2**).

(ii) To characterise the potential risks of microplastics to Galapagos marine invertebrates via priority scoring analysis (**Chapter 3**).

(iii) To compare plastic contamination and its association with macroalgae between the Western and South-Central Biogeographic Zones (**Chapter 4**).

(iv) To use student citizen science data to identify spatiotemporal patterns in beach microplastics (**Chapter 5**).

A visual representation of the six chapters in this thesis are presented in Fig. 1.4 in the format of a traditional Environmental Risk Assessment conceptual framework.

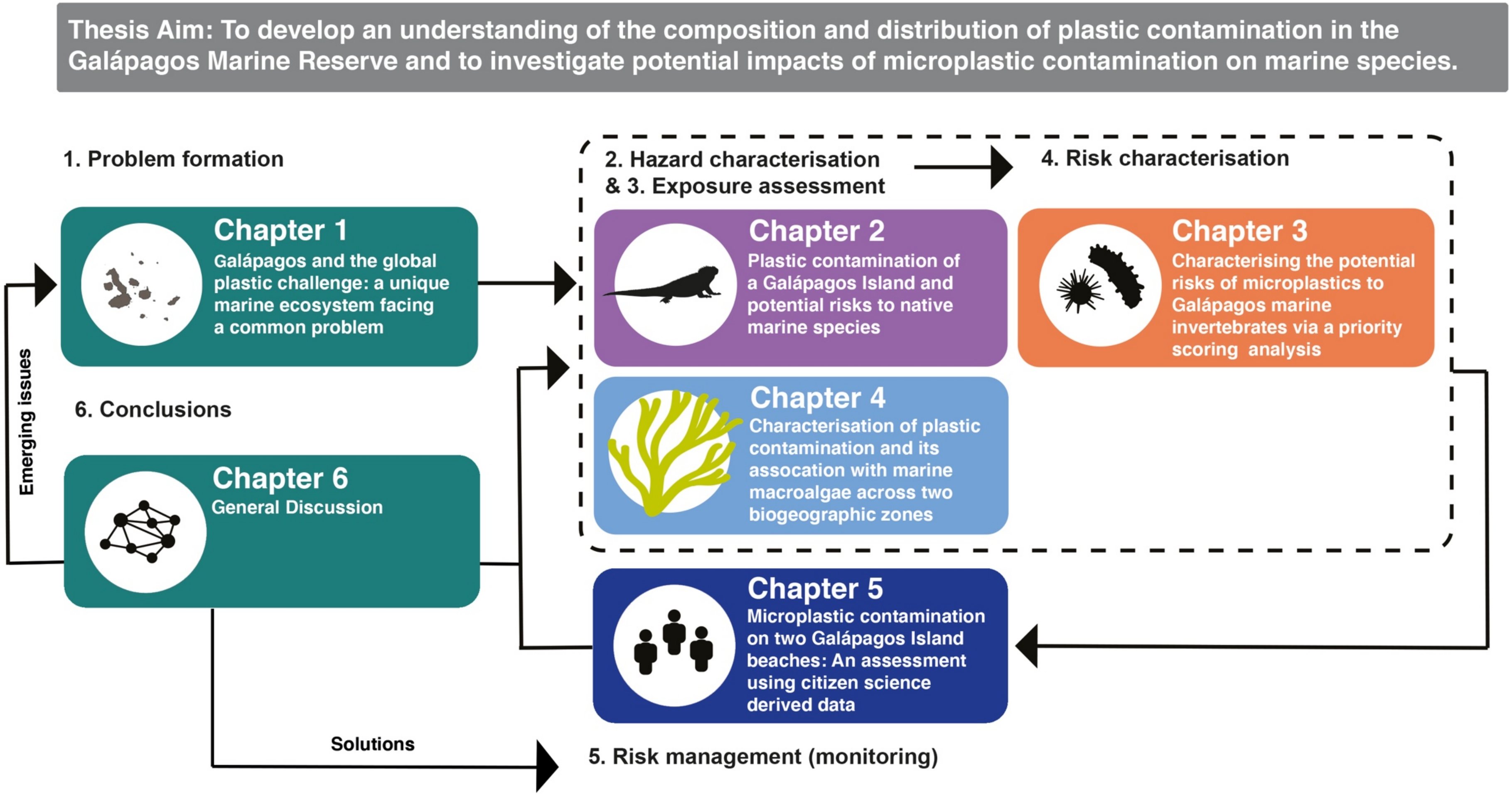

Figure 1.4: Overview of the six chapters of this thesis within a conceptual Environmental Risk Assessment framework

(based on the generic Environmental Risk Assessment described in the European Commission Regulation No. 1829/2003 (via EFSA, 2010))

Chapter 2

Plastic contamination of a Galápagos Island (Ecuador) and potential risks to native marine species

This chapter is included as a PDF of the published manuscript:

Jen S. Jones, Adam Porter, Juan Pablo Muñoz-Pérez, Daniela Alarcón-Ruales, Tamara S. Galloway, Brendan J. Godley, David Santillo, Jessica Vagg and Ceri N. Lewis (2021). Plastic contamination of a Galápagos Island (Ecuador) and potential risks to native marine species. *Science of the Total Environment, 789, https://doi.org/10.1016/j.scitotenv.2021.147704.*

Contributions: J.J., A.P. and C.L. undertook the field sampling campaign and research permits and field logistics were provided by J.P.M.P. and D.A.R..
T.G. and B.G. advised on project design and analysis throughout.
D.S. supported FT-IR analysis and training and J.V. supported priority scoring analysis.
J.J. wrote the main manuscript text with input from A.P., C.L., B.G. and T.G..
J.J. produced the figures, and all authors reviewed the final manuscript.

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

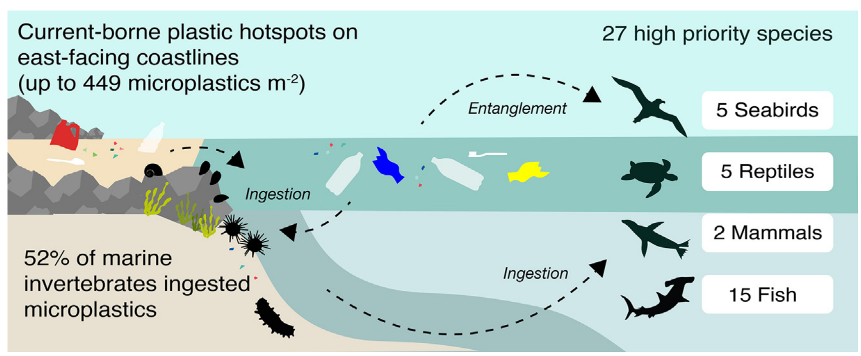
Jen S. Jones [a,b], Adam Porter [a], Juan Pablo Muñoz-Pérez [c,d], Daniela Alarcón-Ruales [c], Tamara S. Galloway [a], Brendan J. Godley [e], David Santillo [f], Jessica Vagg [e], Ceri Lewis [a,*]

[a] Biosciences, College of Life and Environmental Sciences, University of Exeter, Exeter EX4 4QD, UK
[b] Galapagos Conservation Trust, 7-14 Great Dover Street, London SE1 4YR, UK
[c] Universidad San Francisco de Quito (USFQ) & UNC-Chapel Hill Galápagos Science Center (GSC), Av. Alsacio Northia, Isla San Cristobal, Galápagos, Ecuador
[d] School of Science and Engineering, University of the Sunshine Coast, Hervey Bay, QLD, Australia
[e] Centre for Ecology & Conservation, University of Exeter, Penryn TR10 9FE, UK
[f] Greenpeace Research Laboratories, School of Biosciences, Innovation Centre Phase 2, University of Exeter, Exeter EX4 4QD, United Kingdom

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$\cdot$m^{-2}, microplastics 0–448.8 particles$\cdot$m^{-2} or 0–74.6 particles$\cdot$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$\cdot$kg^{-1}) and surface seawater (0.04–0.89 particles$\cdot$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.

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

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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

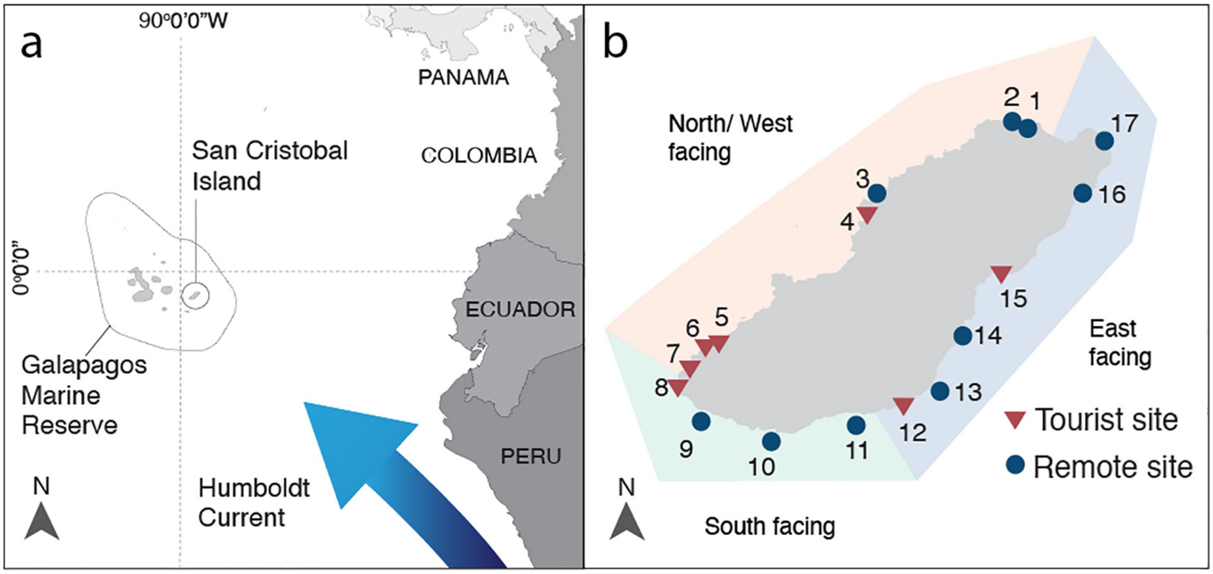

Fig. 1. Geographic location of study site: San Cristobal Island, Galapagos, Ecuador. (a) Geographical location of San Cristobal Island in the Eastern Pacific Ocean showing the Humboldt Current and the limits of the protected Galapagos Marine Reserve; (b) study sites coded by type (tourist sites/remote 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 Perkin-Elmer (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 $\Delta AIC \le 2$ 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 =

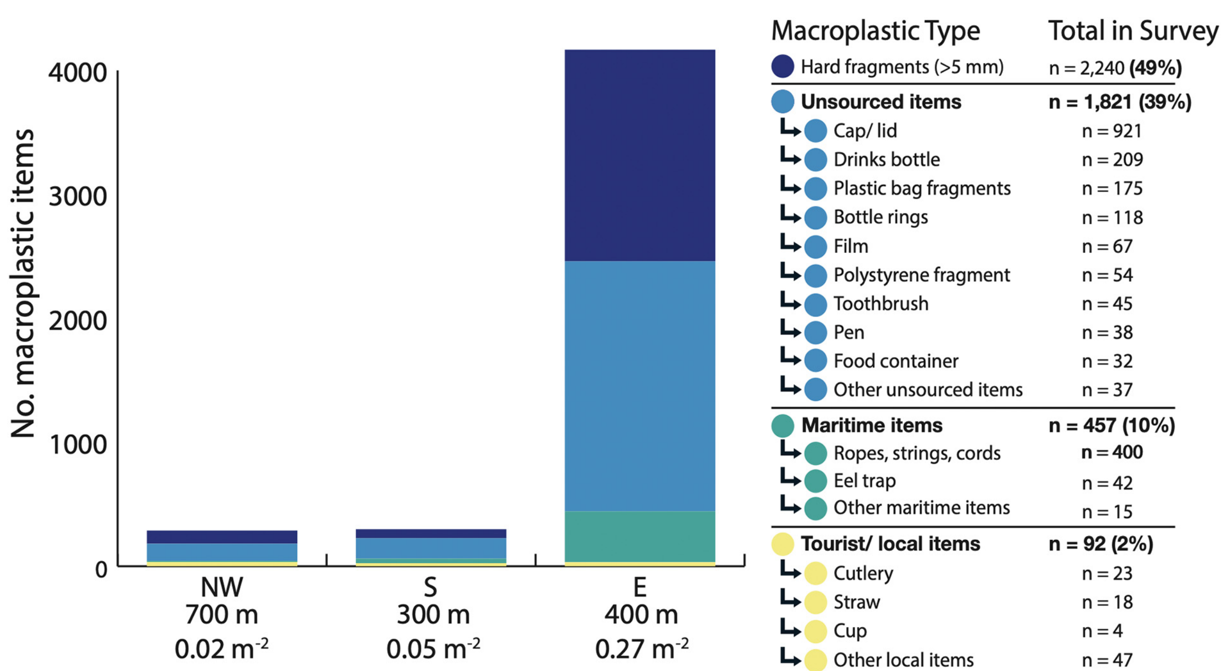

Fig. 2. Composition of beach macroplastic found on San Cristobal Island, Galapagos, Ecuador. Items recovered from the beach surface across 14 north/west, south and east facing beaches (NW, S, E) with total distance surveyed (m) and mean litter density (items·m^{-2}) labelled for each group. Totals and 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$\cdot$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$\cdot$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$\cdot$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$\cdot$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$\cdot$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$\cdot$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$\cdot$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$\cdot$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

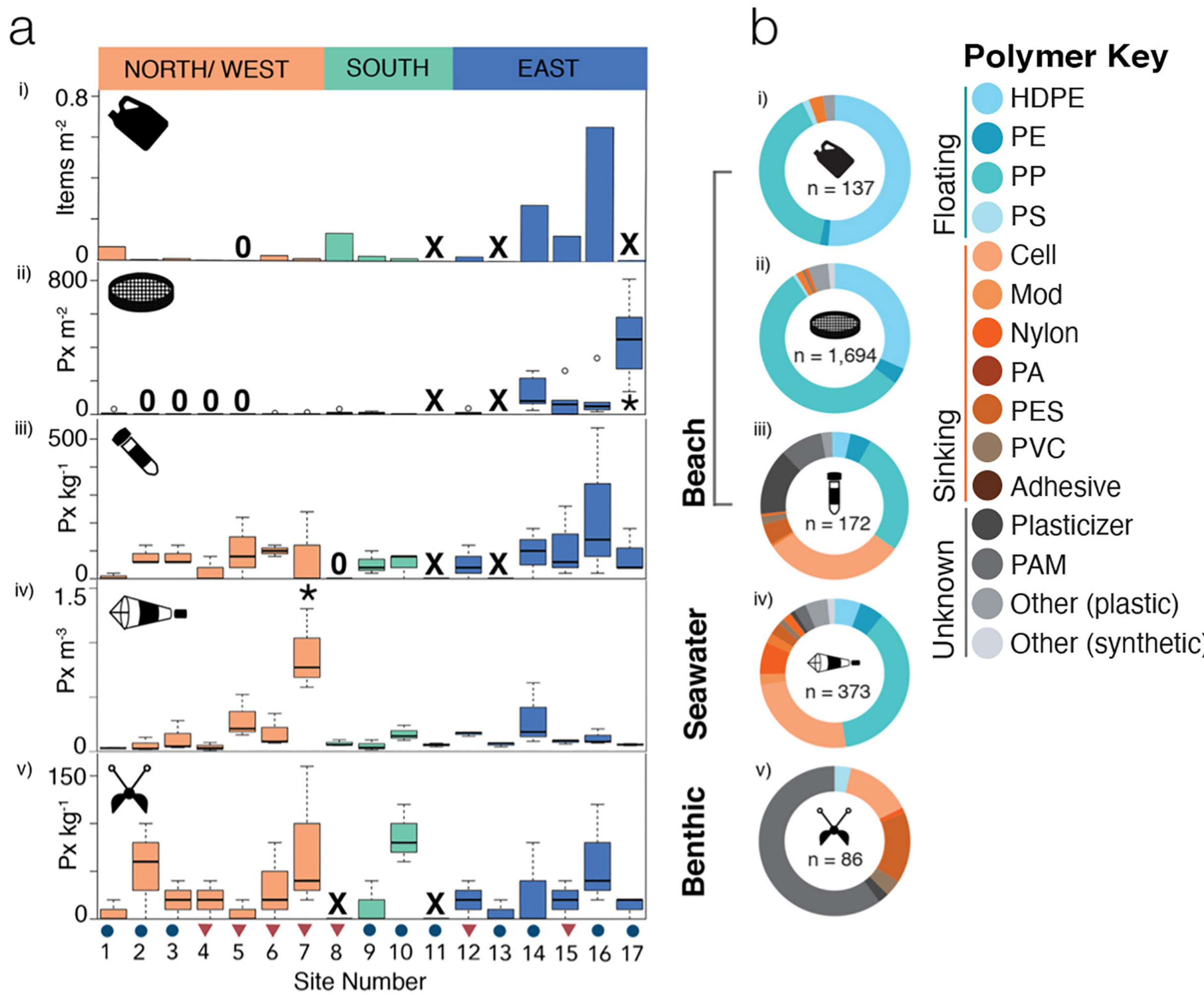

Fig. 3. Abundance and polymer composition of macroplastic items and synthetic particles (including microplastics) around San Cristobal Island, Galapagos, Ecuador. (a) Abundance of (i) beach surface macroplastic (items$\cdot$m^{-2}); (ii) sieved large microplastics (1–5 mm) particles (hereby denoted by px) m^{-2}); (iii) beach sand (px$\cdot$kg^{-1}); (iv) seawater surface (px$\cdot$m^{-3}); (v) benthic sediment (px$\cdot$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 $\pm$ 386 items$\cdot$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 $\pm$ 6.8 particles$\cdot$kg^{-1}, (range 6.7–86.7 particles$\cdot$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$\cdot$kg^{-1}. There are reports of much greater deep sea microplastic concentrations such as 13,600 particles$\cdot$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

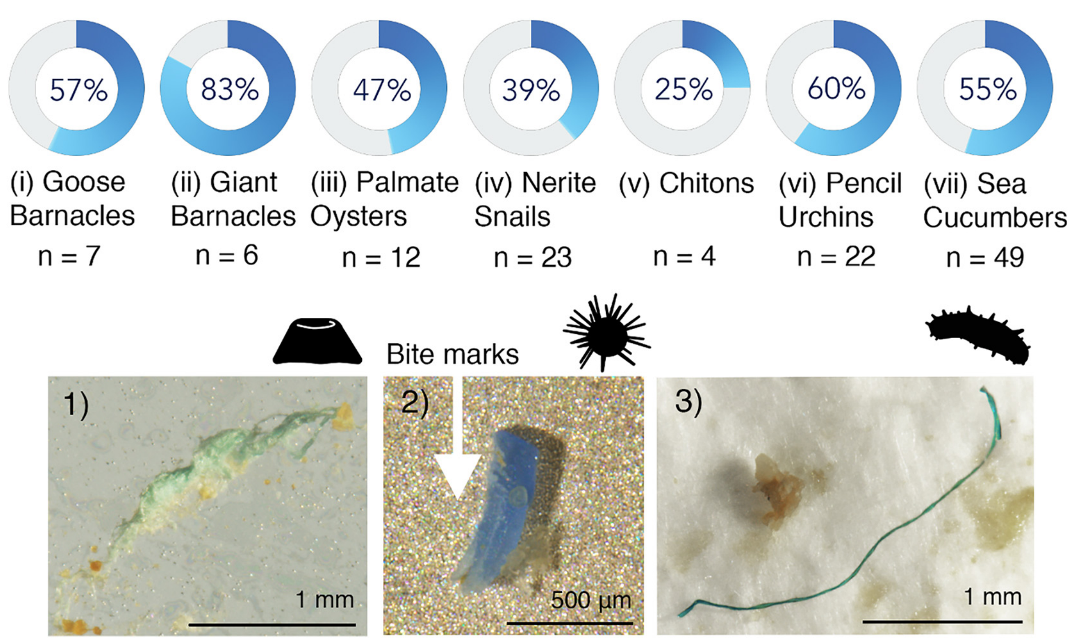

Fig. 4. Synthetic particle uptake in marine invertebrates. Percentage of organisms containing synthetic particles (including petrochemical microplastics and anthropogenic cellulosics) and number of individuals sampled (n) across seven species: (i) goose barnacles (*Lepas anatifera*), (ii) giant barnacles (*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.

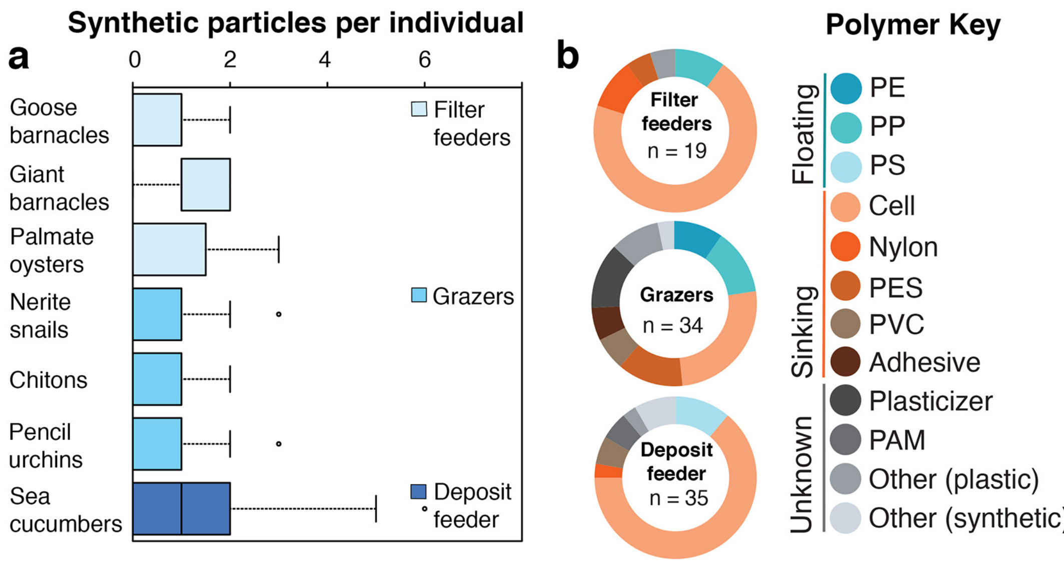

Fig. 5. Synthetic particle abundance and composition in marine invertebrates. (a) Mean synthetic particles per individual averaged over all sampled species grouped by feeding mode; (b) polymer composition of particles (denoted by n) extracted from filter feeders, grazers and surface deposit feeders. 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

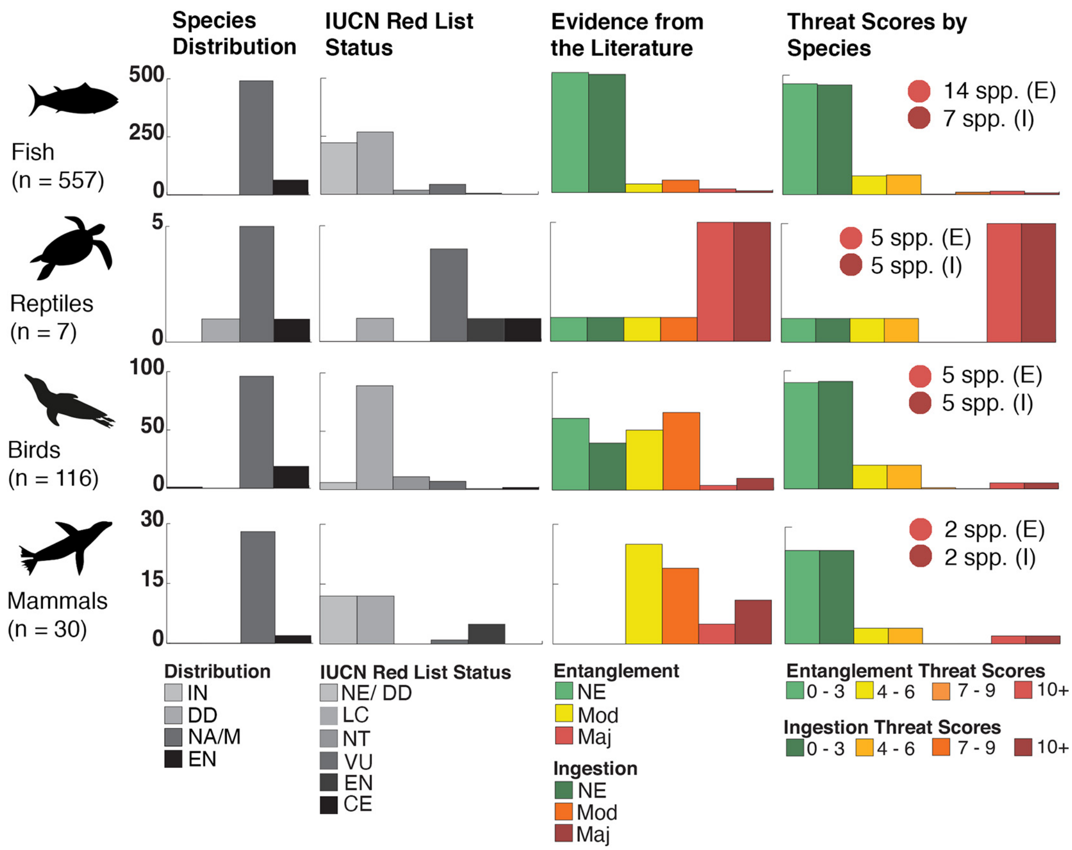

Fig. 6. Summary of priority scoring analysis for Galapagos marine species and plastic contamination. Scoring elements include species distribution, IUCN Red List status and evidence from the literature for harm from plastic contamination caused by entanglement and ingestion at a taxonomic family level. 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

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2021.147704.

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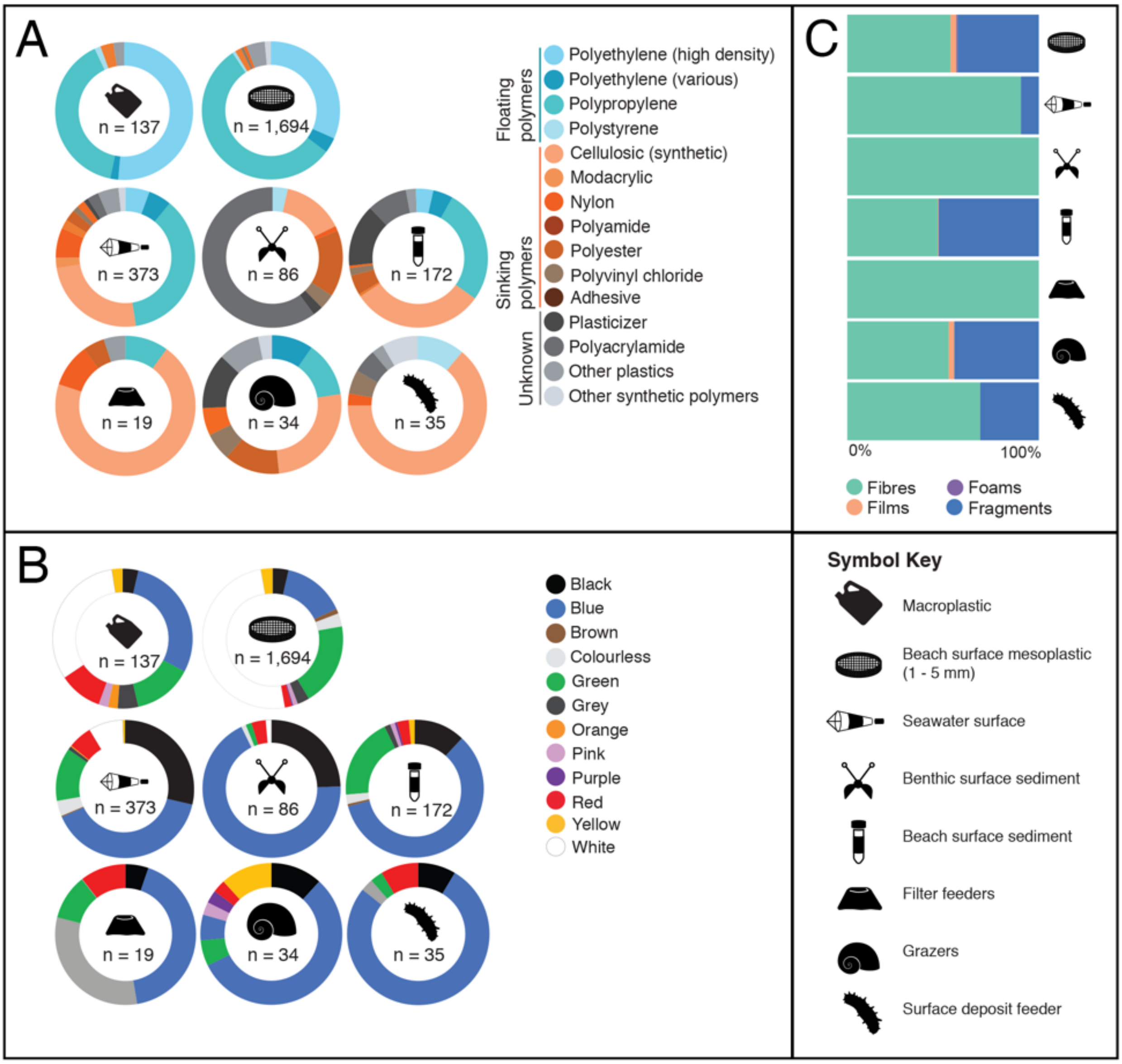
2.2: Supplementary Materials

Supplementary Table 2.1: Site Descriptions (San Cristóbal island)

Site No.	Site Name	Latitude (digital)	Longitude (digital)	Beach area (km2)	Usage	Distance from port (km)	Aspect	Wind	Grain	Beach Description
1	Tuna pequeña	-0.69189	-89.32018	1733	Remote	52	NW	Leeward	Fine	White sand small beach
2	Lobería de Punta Pitt	-0.68731	-89.33844	1033	Remote	50	NW	Leeward	Course	White sand small beach
3	Cerro Brujo Amarillo	-0.7664	-89.45976667	1667	Remote	33	NW	Leeward	Fine	High olivine content of sediment
4	Cerro Brujo	-0.754966667	-89.80916667	4033	Tourist	29	NW	Leeward	Fine	Large white sand beach
5	Baquerizo	-0.88277	-89.59807	2133	Tourist	4	NW	Leeward	Medium	Small mixed sediment beach
6	Carola	-0.89028	-89.61216	2633	Tourist	1	NW	Leeward	Fine	Mixed sediment beach
7	Puerto Baquerizo Harbour/ Playa Mann	-0.900066667	-89.61251667	3500	Tourist	Port (0)	NW	Leeward	Fine	Very small, steep beach, sand to waterline
8	Tongo Reef	-0.908216667	-89.62815	1200	Tourist	2	South	Windward	Fine	Sandy pockets between lava
9	Lobería	-0.92662	-89.61188	2400	Tourist	4	South	Windward	Fine	Large sandy beach between lava rocky shore
10	Matambre	-0.94694444	-89.55237	2033	Remote	12	South	Windward	Course	Mixed sediment beach
11	El Pescador	-0.94694444	-89.42111111		Remote		South	Windward	N/A	N/A
12	Puerto Chino	-0.9268	-89.42693333	4067	Tourist	26	East	Windward	Medium	White sand small beach
13	Churo de Agua Dulce	-0.940083333	-89.479		Remote		East	Windward	N/A	N/A
14	Montones de Arena	-0.857816667	-89.36908333	2533	Remote	37	East	Windward	Fine	Raised back sandy back beach
15	Rosa Blanca	-0.828	-89.35959	4133	Tourist	42	East	Windward	Fine	Sandy pocket between coral rubble bays and rocky outcrops
16	Puerto Tablas	-0.74883	-89.26563	3833	Remote	56	East	Windward	Fine	Raised back sandy back beach, mostly rocky coastline
17	Punta Pitt	-0.709948	-89.253564	3867	Tourist	66	East	Windward	Medium	Sandy beach with high Olivine content

Supplementary Table 2.2: Macroplastic item counts and categories used to complement OSPAR methods. Items were only categorised as sourced from beach visitors/ local litter if they were found on tourist beaches.

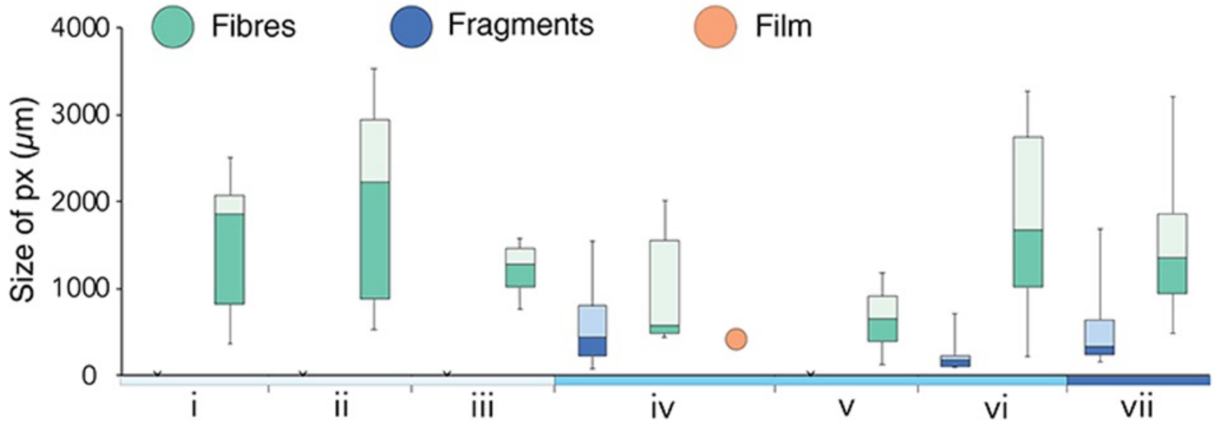
Rank	Macroplastic	Source	Common material	No	%
1	Fragment > 0.5 mm	UNSOURCED	Polyethylene & Polypropylene	2240	0.49
2	Cap/Lid	UNSOURCED	Polypropylene	921	0.20
3	Ropes, Strings, Cords	FISHING/ SHIPPING	Polypropylene	400	0.09
4	Drinks Bottle	UNSOURCED	Poly(ethylene terephthalate) (PET)	209	0.05
5	Plastic Bag Fragment	UNSOURCED	Polyethylene	175	0.04
6	Bottle Ring	UNSOURCED	Polypropylene	118	0.03
7	Cutlery	BEACH/ LOCAL	Polypropylene	77	0.02
8	Film	UNSOURCED	Unidentified Plastic	67	0.01
9	Polystyrene Fragment >0.5mm	UNSOURCED	Polystyrene	54	0.01
10	Toothbrush	UNSOURCED	Unidentified Plastic	45	0.01
11	Eel Trap	FISHING/ SHIPPING	Unidentified Plastic	42	0.01
12	Straw	BEACH/ LOCAL	Polypropylene	40	0.01
13	Pen	UNSOURCED	Unidentified Plastic	38	0.01
14.5	Cup	BEACH/ LOCAL	Polypropylene	32	0.01
14.5	Food Container	BEACH/ LOCAL	Unidentified Plastic	32	0.01
16	Crisp/Sweet Packet and Lolly Sticks	BEACH/ LOCAL	Unidentified Plastic	16	0.00
17	Shoe	BEACH/ LOCAL	Unidentified Plastic	14	0.00
18	Balloon	BEACH/ LOCAL	Styrene Copolymer	12	0.00
19	Rubber O Ring	UNSOURCED	Synthetic Rubber	10	0.00
20.5	Cigarette Lighter	BEACH/ LOCAL	Unidentified Plastic	8	0.00
20.5	Comb/ Hairbrush	UNSOURCED	Unidentified Plastic	8	0.00
22	Rubber Glove	FISHING/ SHIPPING	Synthetic Rubber	7	0.00
24	Black Pipe	UNSOURCED	Unidentified Plastic	6	0.00
24	Floats and Buoys	FISHING/ SHIPPING	Polystyrene	6	0.00
24	Small Plastic Bags	BEACH/ LOCAL	Polyethylene	6	0.00
26	Cosmetics Bottle	FISHING/ SHIPPING	Unidentified Plastic	5	0.00
27.5	Miscellaneous	UNSOURCED	Unidentified Plastic	4	0.00
27.5	Plate	BEACH/ LOCAL	Unidentified Plastic	4	0.00
31	Battery Packet	BEACH/ LOCAL	Synthetic Rubber	2	0.00
31	Cigarette Butts	BEACH/ LOCAL	Cellulose Acetate	2	0.00
31	Plastic Tape	BEACH/ LOCAL	Unidentified Plastic	2	0.00
31	Styrofoam Container	BEACH/ LOCAL	Polystyrene	2	0.00
31	Tangled Nets/Cords/Strings	FISHING/ SHIPPING	Polypropylene	2	0.00
34	Battery	BEACH/ LOCAL	Unidentified Plastic	1	0.00
35.5	Clothes - Baseball Cap	BEACH/ LOCAL	Unidentified Plastic	1	0.00
35.5	Nets and Pieces of Net >0.5mm	UNSOURCED	Polypropylene	1	0.00
35.5	Toys	BEACH/ LOCAL	Unidentified Plastic	1	0.00



Supplementary Figure 2.1:Particle descriptions extracted from environmental media and marine invertebrates around San Cristóbal including polymer composition (A), colour composition (B) and shape (C).

Supplementary Table 2.3: Summary of particles extracted from marine invertebrates in San Cristóbal, Galápagos. Number of individual marine invertebrates sampled from sites with mean no. particles per individual for each site and the overall mean used for the species, size range of ingested particles (see also Supplementary Figure 2.2) and total number of synthetic particles identified.

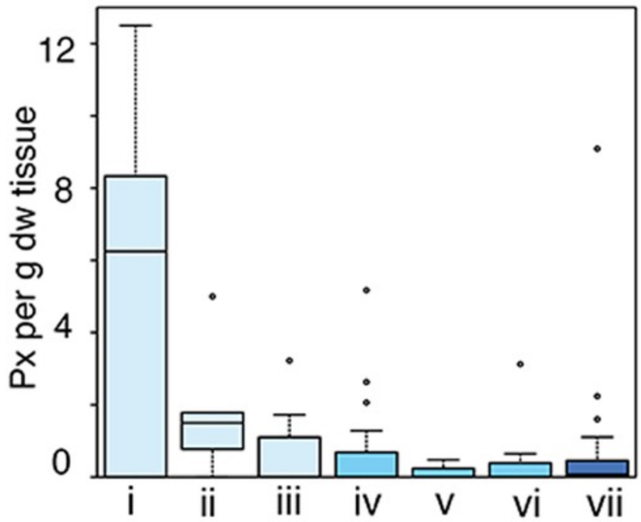
Species	**No. individuals**	**Mean no. particles per ind.**	**Size range (µm)**	**Synthetic particle(s) identified**
Goose Barnacle *Lepas anatifera*	7 from Punta Pitt	0.71	367.1 – 2508.1	2
Giant Barnacle *Megabalanus peninsularis*	6 from Punta Pitt	1.17	519.1 – 8348.3	3 0 7 3 4
Palmate Oyster *Saccostrea palmula*	5 from Lobería de Punta Pitt 7 from Puerto Grande	0.14 1.20 *0.67 overall mean*	733.5 – 1584.87 12572.44 (1 very long fibre)	6 9
Snail *Nerita scabricosta*	11 from Montones 12 from Rosa Blanca	0.36 0.92 *0.64 overall mean*	250.7 – 1876.1 83.5 – 2016.6	4 11
Chiton *Chiton sulcatus*	4 from Lobería de Punta Pitt	0.5	353.4 – 2003.4	2
Galápagos Pencil Urchin *Eucidaris thouarsii*	5 from Montones 5 from Punta Pitt (C) 5 from Punta Pitt (D) 5 from Carola 5 from Rosa Blanca	0.6 1.4 0.6 0 0.8 *0.68 overall mean*	166.4 – 2739.3 106.5 – 2121.8 109.5 – 1015.9 0 1252.6 – 3270.9	3 8 3 0 4
Sea Cucumber *Holothuria kefersteini*	6 from Montones 5 from PPC 5 from PPD 11 from Rosa Blanca	0.4 0.2 0.8 2.55 *0.99 overall mean*	165.5 – 952 828.7 (1 particle) 346.2 – 2243.3 167.7 – 3213.2	2 1 4 **28**



Supplementary Figure 2.2: Sizes (μm) of particles extracted from marine invertebrates in San Cristóbal, Galápagos by particle shape and 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*).

Supplementary Table 2.4: Summary results of negative binomial generalised linear model (GLM) for microplastics ingested by marine invertebrates collected from San Cristóbal, Galápagos, Ecuador. Explanatory variables (out of Species, Feeding Mode, Grain Size, Distance from Port, Site Usage) included for the best fit model. AIC - Akaike's Information Criterion used in the step-wise ranking of models and OD = overdispersion as calculated for the model.

Count data	Explanatory variables	Estimate	Std Error	Z value	*p* value	AIC	OD
Particles contained per individual	Intercept Feeding mode Aspect	-1.423 0.456 0.261	0.607 0.276 0.187	-2.344 1.654 1.399	0.019 0.098 0.162	267.33	1.08



Supplementary Figure 2.3: Synthetic particles extracted from marine invertebrates in San Cristóbal, Galápagos per gram of dry weight tissue across 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*).

Supplementary Table 2.5. **Scoring criteria** including for species distribution, IUCN Red List status, entanglement literature and ingestion literature categories.

Score	**0**	**1**	**2**	**3**
Species Distribution	Invasive	Migratory Unknown	Native	Endemic
IUCN Red List Status		Data Deficient Not Evaluated Least Concern	Near Threatened Vulnerable	Endangered Critically Endangered
Entanglement Literature		No Evidence	Moderate	Major
Ingestion Literature		No Evidence	Moderate	Major

Supplementary Table 2.6: Scores for four example species to demonstrate scoring mechanism (see Supp. Table 2.5 for scoring criteria).

Species	**Distribution**	**Conservation**	**Evidence**	**Total Scores**
Galápagos sea lion *Zalophus wollebaeki*	*3 (endemic)*	*3 (endangered)*	*E = 3; I = 2*	*E = 27; I = 18*
Green sea turtle *Chelonia mydas* (Galápagos subpopulation)	*2 (native)*	*3 (endangered)*	*E = 3; I = 3*	*E = 18; I = 18*
Scalloped hammerhead *Sphyrna lewini*	*2 (migratory)*	*3 (critically endangered)*	*E = 3; I = 1*	*E = 18; I = 6*
Yellowfin tuna *Thunnus albacares*	*2 (migratory)*	*2 (near threatened)*	*E = 2; I = 1*	*E = 8; I = 4*

Supplementary Table 2.7: Table of the 27 Galápagos marine vertebrate species scoring > 10 points in the plastic risk priority scoring analysis, scores indicated by *E* (entanglement) and *I* (ingestion). Endemism status was determined using the Charles Darwin Foundation DataZone (https://www.darwinfoundation.org/en/datazone) and conservation status and unreferenced population estimates were retrieved from the IUCN Red List (https://www.iucnredlist.org/, retrieved 09/01/2020).

Top Scoring Species	Species Distribution	Conservation Status	Example Evidence from the Literature
Galápagos sea lion *Zalophus wollebaeki* *E = 27* *I = 18*	Endemic to Galápagos in terms of established breeding population, large colony resident in harbour of Puerto Baquerizo, San Cristóbal.	Endangered Population estimate 9,200 – 10,600	**Entanglement** 251 Galápagos sea lions reported entangled in plastic fishing gear between 1995-2003 (Alava & Salazar, 2006). Multiple studies into entanglement interactions with closely related species Californian sea lion (*Zalophus californianus*) and Steller sea lion (*Eumetopias jubatus*) from 1976 – 2005 (Hanni & Pyle, 2000; E. Moore *et al.*, 2009). **Ingestion** 194 Steller sea lions recorded ingesting longline fishing gear in (85% juveniles) between 2000 and 2007 (Raum-Suryan *et al.*, 2009).
Galápagos fur seal *Arctocephalus galapagoensis* *E = 27* *I = 18*	Endemic to Galápagos, generally restricted to remote areas in the western and central islands.	Endangered Population estimate 10,000	**Entanglement** Multiple studies into entanglement interactions with closely related species the northern fur seal (*Callorhinus ursinus*) and Antarctic fur seal (*Arctocephalus gazella*) 1980 – 2015 mostly with fishing litter such as monofilament netting and lines (Lawson *et al.*, 2015; McIntosh *et al.*, 2015). **Ingestion** 7% Arctocephalus australis (n = 133) had ingested large fragments of plastic bags and fishing gear (Denuncio *et al.*, 2017).
Waved albatross *Phoebastria irrorata* *E = 18* *I = 27*	Endemic to Ecuador in terms of breeding population (forages in the Eastern Pacific outside of the Galápagos Marine Reserve boundary). Nesting colonies on Española island.	Critically Endangered Population estimate >34,694 in 2001 (Anderson *et al.*, 2002)	**Entanglement** A 2018 review of bird entanglement in plastic and other synthetic materials reports records for 12 out of 21 albatross species(Ryan, 2018). **Ingestion** 3 out of 40 dead waved albatross chicks contained suspected plastics in a 2007 survey in Galápagos (Anderson *et al.*, 2008). During necropsies of 45 black-browed albatrosses (*Thalassarche melanophris*) and 26 Atlantic yellow-nosed albatrosses (*Thalassarche chlororhynchos*), 90% of birds sampled had ingested anthropogenic items and plastic represented 85% of debris found in birds' stomachs (Colabuono & Vooren, 2007).

Galápagos petrel *Pterodroma phaeopygia* *E = 18* *I = 18*	Endemic to Ecuador in terms of breeding population (forages in the Eastern Pacific). Breeding colonies in Floreana, San Cristóbal and Santa Cruz islands in Galápagos.	Critically Endangered Population estimate 6,000 – 15,000	**Entanglement** 23% giant petrels (*Macronectes giganteus*) (7 individuals) over two seasons recorded as entangled in net/ rope – presumed to be debris as opposed to interactions with long-line gear in use (Phillips *et al.*, 2010). **Ingestion** Non-lethal sampling (emetic) of Leach's storm petrels (*Oceanodroma leucorhoa*) yielded > 50% sample size had ingested plastic and were witnessed giving plastic to offspring (Bond & Lavers, 2013). A study of southern giant petrels reported as much plastic as cephalopods and fish in the stomachs of birds (Petry *et al.*, 2010).
Galápagos penguin *Spheniscus mendiculus* *E = 18* *I = 18*	Endemic to Galápagos, primary colonies in the western Archipelago but occasional sightings in the central islands and islets.	Endangered Population estimate 1,200	**Entanglement** Entanglement records for 7 spheniscid penguin species globally, primarily with fishing litter (Ryan, 2018). **Ingestion** A related species, Magellanic penguins (*Spheniscus magellanicus*), in Brazil were found to have obtained serious injury or death from plastic. Out of 175 birds, 26 had ingested plastic debris, which could have led to death (Brandão *et al.*, 2011).
Flightless cormorant *Phalacrocorax harrisi* *E = 18* *I = 12*	Endemic to Galápagos, restricted to Isabela and Fernandina islands in the western Archipelago.	Vulnerable Population estimate 2,080 individuals in 2013 (Jiménez-Uzcátegui, 2013)	**Entanglement** 47 cases of entanglement of Brandt's cormorant (*Phalacrocorax penicillatus*) have been reported by citizen scientists in California 1997 – 2017 mostly in discarded fishing line (Donnelly-Greenan *et al.*, 2019). **Ingestion** Pied cormorant (*Phalacrocorax varius*) 5 out of 22 birds had ingested plastic including fishing gear (Roman *et al.*, 2016).
Lava gull *Leucophaeus fuliginosus* *E = 12* *I = 12*	Endemic to Galápagos, frequently sighted on beaches at study sites in San Cristóbal.	Vulnerable Population estimate 600 - 800	**Entanglement** Study in California on a variety of gull and pelican species, noted frequent fishing net related injuries to all species with higher frequency in the summer months (Dau *et al.*, 2009). 22 freshly dead kelp gulls *Larus dominicanus* were reported on a 9-day survey of 6km beach in Argentina due to entanglement in monofilament fishing line (Yorio *et al.*, 2014). **Ingestion** Ingestion of fishing hooks and associated plastic debris (primarily monofilament lines) from recreational fisheries was commonly reported in Korea in black tailed gulls (*Larus ridibundus*) - ingestion of the hooks was also linked to entanglement in the trailing line sometimes completely preventing movement and resulting in death (Hong *et al.*, 2013).
Green sea turtle *Chelonia mydas* (previously known as *Chelonia agassizii*)	Native, >40% of Eastern Pacific population in Galápagos with important turtle nesting beaches on	Endangered Population unknown	**Entanglement** Global evidence for frequent green turtle entanglement in plastic debris and of mortality in ghost fishing nets as a result of illegal fishing practices as reviewed by Duncan *et al.* (Duncan *et al.*, 2017). **Ingestion** Multiple studies evidence plastic debris affecting gut function and feeding behaviour, leading to death. A quantitative analysis linked a 50% probability of mortality with sea turtles that ingested >14 pieces of plastic

E = 18 *I = 18*	several islands (Seminoff, 2007).		(Wilcox *et al.*, 2018). Common items found in stomachs include plastic bags, plastic ropes and hard plastic pieces.
Hawksbill turtle *Eretmochelys imbricate* *E = 18* *I = 18*	Native but uncommon in Galápagos.	Endangered Population unknown	See Green sea turtle
Marine iguana *Amblyrhynchus cristatus* *E = 12* *I = 12*	Endemic to Galápagos, possibly up to 11 sub-species (Miralles *et al.*, 2015).	Vulnerable Population unknown due to taxonomic uncertainty	No family level evidence, using green sea turtle evidence as a proxy due to algae diet and use of similar nesting sites.
Whale shark *Rhincodon typus* *E = 18* *I = 18*	Migratory, most sightings July – October	Endangered Population unknown	**Entanglement** Whale shark entanglements were the most frequently reported on Twitter in a study primarily in fishing gear (Parton *et al.*, 2019). **Ingestion** Plastic debris including packing straps, food wrappers, a disposable cup and a cigarette butt recovered from gills and gut of stranded whale shark in the Philippines (Abreo *et al.*, 2019).
Scalloped hammerhead shark *Sphyrna lewini* *E = 18* *I = 18*	Migratory, nursery grounds confirmed around San Cristóbal Island	Critically Endangered Population unknown	**Entanglement** Hammerhead entanglement in fishing gear was reported frequently on Twitter (Parton *et al.*, 2019). **Ingestion** Most likely to be exposed via trophic transfer but no evidence found.
Open water sharks considered to have similar risks to scalloped hammerhead: tiger (*Galeocerdo cuvier*), blue (*Prionace glauca*), great hammerhead (*Sphyrna mokarran*), pelagic thresher (*Alopias pelagicus*), blacktip (*Carcharhinus limbatus*), whitetip (*Triaenodon obesus*), oceanic whitetip (*Carcharhinus longimanus*), grey reef (*Carcharhinus amblyrhynchos*), sandbar (*Carcharhinus plumbeus*), silky (*Carcharhinus falciformis*), shortfin mako (*Isurus oxyrinchus*).			
Salema spp. *Xenichthys agassizii* & *Xenocys jessiae* *E = 12* *I = 6*	Endemic to Galápagos		**Entanglement** Entanglement in plastic debris reported in tropical reef fish is predicted to cause movement restrictions and affect feeding and predator avoidance behaviours (Nunes *et al.*, 2018). **Ingestion** N/A

Chapter 3

Characterising the potential risks of microplastics to Galápagos marine invertebrates via a priority scoring analysis

Abstract

Marine invertebrates perform critical roles in ecosystem functioning in the Galápagos Marine Reserve, representing a major part of threatened marine vertebrate diets, and also having major socioeconomic importance as an important contingent of the artisanal fishery. Despite this importance, marine invertebrates are severely understudied in Galápagos with the majority of biological research and conservation efforts focused on charismatic, threatened vertebrate species. Pollution is linked with the declines of >80% of invertebrate populations that have been assessed so far for the IUCN Red List and the growing presence of microplastic contamination across marine habitats raises concerns about the potential ecological and socioeconomic impacts, necessitating some form of risk assessment, particularly for keystone and commercial species. Here I adapt the priority scoring analysis applied to vertebrates in **Chapter 2**, combining species distribution information, IUCN Red List conservation status, commercial importance and literature evidence of harm from ingestion of plastics in similar species to calculate a priority score (S^P), and investigate if this approach is a useful tool to direct research and mitigation for invertebrates despite the lack of data. Out of 2,449 marine invertebrate species recorded in the Archipelago that were assessed, 15 priority species were identified via this approach (S^P > 5, maximum 81), with the highest scoring being the brown sea cucumber (*Isosthichopus fuscus*) (S^P = 18). The remaining 14 priority species had an S^P between six and nine and included three corals, six decapod crustaceans (three of which are commercially exploited), two chitons (both commercially exploited), two mussels and a clam. Over 99% of species had an S^P < 4 due to lack of distribution and conservation status

information highlighting major fundamental knowledge gaps. This scoring mechanism highlights some invertebrate species of conservation interest but insufficient data are available to be as effective as for vertebrates where impacts are easier to observe. In the context of these results, I offer some recommendations for further research priorities to address these knowledge gaps for this important group at the foundation of the Galápagos marine food web.

3.1. Introduction

The identification of species most at risk from environmental pollutants can be challenging, often necessitating some form of risk assessment to target research and mitigation action. The role of a contaminant risk assessment in environmental management and policy is to minimise threats to human health, to ensure healthy ecosystem function and to ensure particular species are conserved for commercial, recreational or biological motivations (Depledge & Fossi, 1994). Formal risk assessment incorporates several stages: (i) the **characterisation of hazard** i.e. the profile of harm, ideally considering response in multiple species across trophic levels and potential multi-stressor effects, (ii) the **exposure likelihood** mapped against relevant pollution pathways, (iii) the **characterisation of risk** i.e. the combination of hazard and exposure information and (iv) the **evaluation of uncertainties** (Werner *et al.*, 2016).

The application of traditional ecotoxicological risk assessments to microplastic contamination has proved difficult and sometimes controversial. Although ubiquitous in the environment, microplastics tend to be found in concentrations that represent a relatively low toxicological risk (at least to vertebrates) but the lack of studies to assess long-term impacts suggests that a precautionary approach should probably be adopted in lieu of more complete data (Backhaus & Wagner, 2020; Koelmans *et al.*, 2017). The environmental burden of microplastic is growing; if predictive modelling is correct, in the best case scenario, plastic waste leakage to the environment will reach 710 million tonnes in the period between 2016 – 2040 i.e. if concerted efforts are made across the plastics supply chain, both ‘upstream’ (reduction and substitution of plastics) and ‘downstream’ (waste disposal and recycling improvements) at a global scale (Lau *et al.*, 2020). Although the engagement of governments, businesses and civil society in tackling plastic pollution is encouraging, action has not been adequate to stem the flow, meaning this best case scenario is highly unlikely in the near future (Barrowclough & Birkbeck, 2020). In addition, the breakdown and fragmentation of the estimated 4,600 million tonnes of plastic matter already accumulated in landfill and the natural environment between 1950 and 2015 (Geyer *et al.*, 2017), represents a substantial and growing environmental burden.

In highly protected areas such as the Galápagos Marine Reserve, it is not possible nor ethical to undertake widespread biotic sampling or to perform ecotoxicological assays on endemic species with small populations. Hence, alternative methodologies are necessary to establish potential risk. The IUCN Red List species vulnerability assessments provide a valuable tool for prioritising research and management to benefit species most at risk of extinction. The IUCN Red List heavily favours higher vertebrates however, probably as the focus of the majority of conservation interest is centred on ‘flagship species’ that are of high public interest (Simberloff, 1998; Zacharias & Roff, 2001) and that larger organisms are generally easier to sample. Invertebrates, fungi and plants are grossly underrepresented, despite these groups representing > 99% of all described species (Stuart *et al.*, 2010). As of December 2020, 2% of the 1,551,836 described invertebrates (including terrestrial species) had been evaluated for the Red List, contrasting with 74% of vertebrates (total 72,906 species). A recent focus on corals has seen 40% of species evaluated resulting in the identification of 237 threatened species but only 11% of molluscs, 4% of crustaceans and 0.55% of all other known marine invertebrate species have been assessed (IUCN, retrieved Feb 2021). Pollution is cited by a review by Collier *et al.* (2016) as a likely contributing factor in population declines of 80% of the IUCN assessed invertebrate species (total = 324 species), second only in severity to the threat of overfishing (Collier *et al.*, 2016).

Invertebrates have high ecological importance across global habitats; making up the majority of animal biomass and providing critical ecosystem services such as nutrient cycling, detrital decomposition, water purification and pollination (Collier *et al.*, 2016). E. O. Wilson famously postulated that 'invertebrates run the world' and the removal of them from the natural environment would likely cause the rapid extinction of most vertebrates, including humans (Wilson, 1987). The 'Linnean shortfall' of invertebrates, i.e. the lag in discovery and taxonomic description, means that species are being lost before we even have the chance to describe them, recently labelled as the 'quiet extinction' of the ocean (Costello *et al.*, 2010; Eisenhauer *et al.*, 2019; Stuart *et al.*, 2010; Whittaker *et al.*, 2005).

Marine invertebrates are keystone species in the rocky reef ecosystem of Galápagos (the predominant coastal habitat) and have major ecological importance (Edgar *et al.*, 2010; Vinueza *et al.*, 2006). The majority of biomass is made up of small filter feeders (mostly barnacles) and gastropods (Okey *et al.*, 2004). Predators such as lobsters and octopus occupy relatively high trophic levels, enforcing top-down control of herbivores such as sea urchins that due to their voracious grazing of macroalgae, have been responsible for major ecological regime shifts, particularly during ENSO events (Vinueza *et al.*, 2014). Invertebrates represent an important contingent of the artisanal fishery (see Table 2) and the overexploitation of spiny lobsters and sea cucumbers have caused substantial conservation concern (Hearn, 2008; Toral-Granda et al., 2008). The combination of their ecological importance, their role as a major part of threatened vertebrate diets and their direct connection with the human food chain mean that delineating the potential risks of pollutants to marine invertebrates is of high priority.

Great strides have been made over the last ten years to describe microplastic uptake pathways in marine invertebrates. These include the direct ingestion of particles, either selectively or due to mistaken identity for normal food items of similar size e.g. plankton or sediment grains (Cole *et al.*, 2015; Graham & Thompson, 2009), indirect ingestion of particles via association with dietary items or trophic transfer (Farrell & Nelson, 2013; Gutow *et al.*, 2016), and also inhalation through the gills (Gray & Weinstein, 2017; Watts *et al.*, 2016). Evidence of uptake in wild specimens has been described in a plethora of species demonstrating high bioavailability across many feeding guilds including copepods (Sun *et al.*, 2017), crabs (Horn *et al.*, 2020; Waddell *et al.*, 2020), barnacles (Goldstein & Goodwin, 2013; Xu *et al.*, 2020), bivalves (Renzi *et al.*, 2018), gastropods (Doyle *et al.*, 2019), sea urchins (Feng, Wang, *et al.*, 2020) and sea cucumbers (Renzi & Blašković, 2020). The elucidation of biological harm caused by different types of microplastics, particularly via their potential role as vectors of POPs (Rochman *et al.*, 2019) and pathogens (Bowley *et al.*, 2021) is a major priority for the field but also an area of high debate (Backhaus & Wagner, 2020; Koelmans *et al.*, 2014). Potential adverse impacts span the sub-cellular to the population scale but frequently these biological end-points have only been measurable in the laboratory with exposure concentrations much higher than those that have been recorded in the environment. Exposure studies often use singular laboratory grade microplastic types e.g. polystyrene spherules that are not reflective of the heterogenous microplastic profile in the environment that incorporate a wide spectrum of polymers, sizes and shapes (Burns & Boxall, 2018; Haegerbaeumer *et al.*, 2019).

The logical first step when considering the risk to a new ecosystem, is to characterise microplastic ingestion over a suite of key species to investigate how widespread uptake is, and to characterise what particles are commonly found. In **Chapter 2**, microplastic uptake is reported in seven marine invertebrate species collected from the coastline of San Cristóbal island including grazers, filter/ suspension feeders and surface deposit feeders. These data, combined with environmental contamination data across relevant habitats enable us to start to define the relevant pathways (the second stage of the risk assessment) but do not help us to define ecotoxicological hazard (the first stage) i.e. the potential harm, past the initial assumption that uptake would be a precursor to adverse effects.

Here the application of a systematic priority scoring analysis for the potential impacts of microplastic ingestion in Galápagos marine invertebrates is tested, with a modified approach based on that developed for vertebrates in **Chapter 2** to investigate if this type of rapid assessment method still represents a useful tool, even for data poor invertebrate species groups. To consider a range of prioritisation factors, the following elements were scored: (i) species distribution (considering endemic species as highest priority), (ii) conservation status (using the IUCN Red List status to highlight documented endangered species), (iii) commercial importance (considering overexploited species as a priority) and (iv) evidence of harm caused by the ingestion of microplastics in similar species (at a taxonomic family level) in the published literature.

3.2. Methods

Priority scoring for marine invertebrates

Species lists for marine invertebrates described in the Galápagos Marine Reserve were retrieved from the Charles Darwin Research Station Natural History Collections database collated from sightings over several decades (www.darwinfoundation.org/en/datazone), incorporating 2,449 species with information on distribution and origin. Nematodes, platyhelminths, ctenophores and microscopic zooplankton fauna (with the exception of copepods) were not considered due to lack of information in all scoring categories. Larval stages of macroinvertebrates were also omitted. Species were given a distribution score (S^D), IUCN Red List statuses was retrieved from the IUCN database (https://www.iucnredlist.org/) to generate a conservation score (S^C) and a commercial importance score ($S^\$$) was applied (see Table 1 for scoring criteria and Table 2 for a list of commercially exploited marine invertebrates).

The definition of ‘harm’ used was that proposed by Nordberg *et al.*, in their ‘Glossary of terms used in ecotoxicology’ for the International Union of Pure and Applied Chemistry, defined simply as an “adverse effect to an ecosystem, community, population, species, individual organism, organ, tissue or cell” (Nordberg *et al.*, 2009). Therefore, according to this definition, effects such as growth impairment and reduced reproduction success would be considered harm. To establish literature evidence of harm from ingestion (S^{IL}) a literature search was undertaken using the Web of Science and Scopus databases incorporating the terms “invertebrate” AND “marine” AND “plastic” OR “microplastic” (retrieved 27 February 2021). After scanning titles, this yielded 60 original studies (not including review articles) related to marine invertebrates and plastic ingestion. Abstracts were screened and a total of 43 were rejected from the scoring analysis due to the following reasons: they showed only uptake and no harm was measured (n = 22), they were methods development focused (n= 2), they described the impacts of nanoplastics < 1 µm (n = 3), they measured harm but no significant results were obtained (n = 6), or they were focused on taxonomic groups or ontogenetic stages not considered in this analysis, namely nematodes (n = 1) or larval planktonic stages of normally benthic organisms such as barnacles or sea urchins (n = 9). To supplement this list, four review papers (Foley *et al.*, 2018; Haegerbaeumer *et al.*, 2019; Pinheiro *et al.*, 2020; Sharifinia *et al.*, 2020) were also mined for references yielding a further 16 studies. Study species were mapped to the Galápagos species list and those focusing on species from taxonomic families not represented in Galápagos were also removed, namely lugworms (Arenicolidae, n = 2), cyclopoid copepods (Cyclopettidae, n = 1), mitten crabs (Varunidae, n = 2) and the purple sea urchin (Perechinidae, n = 1), resulting in a final count of 27 studies describing demonstrable harm from microplastic ingestion for inclusion in the scoring analysis (Table 3.3).

Unlike in the vertebrate scoring, it was not feasible to search for each genus due to the number of species and thus the scoring criteria were modified to include evidence found at a family level within species of the same feeding mode. Evidence of harm from ingestion in the literature was scored as follows: no literature evidence or no significant effect (1), moderate evidence e.g. harm measured but not at environmentally relevant concentrations (2) and major evidence e.g. harm measured at environmentally relevant concentrations (3) (Table 3.1). Upper 'environmentally relevant' concentrations for microplastics in sediment are defined as < 3% dry weight (after Carson *et al.*, 2011) or 500 particles kg^{-1} (after Ziajahromi *et al.*, 2018). For seawater, an upper concentration of 0.29 µg L^{-1} (Shi *et al.*, 2020) or 100 particles L^{-1} reflecting the highest particle counts reported to date from a polluted harbour in Norway (after Noren & Naustvoll, 2010), acknowledging that this is much higher than reported to date elsewhere and that the criteria of 'environmentally relevant' concentrations far exceed those measured in Galápagos to date (see **Chapter 2**).

The final equation to establish the priority score (S^P) was as follows:

$$S^P = S^D \times S^C \times S^{\$} \times S^{IL}$$

Table 3.1: Scoring criteria including for species distribution, IUCN Red List status, commercial importance and ingestion literature.

Score	0	1	2	3
Distribution **(S^D)**	Invasive	Migratory Unknown	Native	Endemic
IUCN Red List Status **(S^C)**		Data Deficient Not Evaluated Least Concern	Near Threatened Vulnerable	Endangered Critically Endangered
Commercial importance **($S^\$$)**		No commercial fishery	Small fishery (likely predominantly local consumption)	Large and overexploited fishery (likely predominantly exported)
Ingestion Literature **(S^{IL})**		No Evidence	Moderate: harm recorded in laboratory exposures exceeding environmentally relevant concentrations*	Major: harm recorded in wild populations or in laboratory exposures at environmentally relevant concentrations*

* Environmentally relevant microplastic concentrations defined as < 3% dry weight or 500 particles kg^{-1} for sediment (Carson *et al.*, 2011; Ziajahromi *et al.*, 2018) and 0.29 μg L^{-1} or 100 particles L^{-1} (Shi *et al.*, 2020) .

Table 3.2: Table of commercially exploited invertebrates in the Galápagos Marine Reserve (sources: IUCN Red List (https://www.iucnredlist.org), SeaLife Base (https://www.sealifebase.ca)).

Species	Distribution	IUCN Red List status	Diet	Fishery information
Crustaceans				
Red spiny lobster *Panulirus penicillatus*	Native (tropical Indo-Pacific region)	*Least Concern*	Predator of benthic invertebrates	**7% locally consumed, 93% exported (inc. *P. gracilis*)** Seasonal dive fishery (September – December). Lobster fishery worth an estimated $1 million per year 2006 – 2012 (more recent estimates not found) (Ramirez *et al.*, 2013; Schiller *et al.*, 2014)
Green spiny lobster *Panulirus gracilis*	Native	*Data Deficient*	Predator of benthic invertebrates	See above
Galápagos slipper lobster *Scyllarides astori*	Native	*Data Deficient*	Predator of benthic invertebrates	**88% locally consumed, 12% exported** (Schiller *et al.*, 2014) Year-round dive fishery until recently but in 2021, the fishery opening has been restricted from March - September when the spiny lobster fishery opens. Commonly available in tourist restaurants.
Molluscs				
Galápagos octopus *Octopus oculifer*	Native	*Not Assessed*	Predator of benthic invertebrates	**Predicted 100% locally consumed** Dive fishery, used as bait in drag fishing for sports fish, also eaten locally
Giant conch *Hexaplex princeps*	Native	*Not Assessed*	Predator of other molluscs	**Predicted 100% locally consumed** Dive fishery
Galápagos giant conch *Pleuroploca princeps*	Native	*Not Assessed*	Predator of other molluscs	**Predicted 100% locally consumed** Dive fishery
Galápagos giant chiton *Chiton goodalli*	Endemic	*Not Assessed*	Algae grazers	**Predicted 100% locally consumed** Small-scale fishery for local and tourist consumption - canchalagua ceviche is a common dish. Numbers estimated in 1999 as 1,000 chitons harvested per month (Herrera *et al.*, 2000). Collected with a knife off intertidal rocks, often at full moon.
Galápagos giant chiton *Chiton sulcatus*	Endemic	*Not Assessed*	Algae grazers	**Predicted 100% locally consumed** As above although *C. goodalli* are more commonly found in primary harvesting locations (Herrera *et al.*, 2000).

Echinoderms				
Brown sea cucumber *Isostichopus fuscus*	Native	*Endangered*	Deposit feeder (surface sediments)	**100% exported** Originally an open-access fishery in the mid 1990s before bans following population collapse due to rapid overexploitation. Fishery reopened in 1999 with a participatory management strategy but with > 80% population decline, has been termed 'economically extinct' since (Toral-Granda et al., 2008). Illegal catches are a recognised problem.
Warty sea cucumber *Stichopus horrens*	Native	*Data Deficient*	Deposit feeder (surface sediments)	**Illegally harvested** Illegal catches of this sea cucumber species, which seems to also form part of the sea cucumber exports (< 1%) as reported in Schiller *et al.*(2014). There were suggestions to legalize the fishery for *S. horrens* given the depletion of I. *fuscus* by overfishing (Castrejon, 2011) but this has not yet happened.
Sea cucumber *Holothuria atra*	Native	*Least Concern*	Deposit feeder (surface sediments)	**Illegally harvested** Scarce or lack of qualitative information and no quantitative data (catches) for this illegally harvested sea cucumber species (Schiller *et al.*, 2014) There are no biological or ecological data for management, as previously reported by Toral-Granda (2008).
Sea cucumber *Holothuria kefersteini*	Native	*Data Deficient*	Deposit feeder (surface sediments)	**Illegally harvested** Lack of qualitative information and no quantitative data for this illegally harvested sea cucumber species (Schiller *et al.*, 2014).
White sea urchin *Tripneustes depressus*	Native	*Not assessed*	Grazer, feeding on macroalgae, sponges and benthic invertebrates	**Illegally harvested** This large sea urchin species is illegally harvested, but its fishery may be legalized in the future (Castrejon, 2011). No data on conservation status and harvests.

3.3. Results

Microplastic ingestion harm evidence in the literature

Of the 27 studies included in the priority scoring analysis (Table 3.3), 47% (n = 15) used bivalve molluscs, primarily *Mytilus* spp. (n = 10). Arthropod studies made up 31% (n = 7), annelid studies comprised 19% (n = 4) and there was one study on a scleractinian coral. Only five studies showed harm in species exposed to environmentally relevant concentrations including increased mortality and reduced reproductive success in crabs exposed to polyethylene fibres and cellular effects such as inflammation of immune cells, oxidative responses and increased cell apoptosis in four bivalves exposed most commonly to polystyrene spherules/ beads (Table 3.3).

Scoring results

Of the 2,449 invertebrate species assessed, only 15 species had an S^P > 5 (out of a maximum of 81), summarised in Table 3.4. The highest scoring was the brown sea cucumber *Isosthichopus fuscus* (S^P = 18), followed by three scleractinian corals (S^P = 7 – 9), five decapod crustaceans and six bivalves (all S^P = 6). Eighty-seven percent of species scored < 2 in this analysis, including all annelids (79 spp.), bryozoans (147 spp.), tunicates (15 spp.) and sponges (40 spp.) and therefore these groups were not included in the summary of results (Fig. 3.1).

Table 3.3: Literature evidence of marine invertebrate harm from microplastics (including sub-cellular, cellular, organism and population level effects) particle type and concentrations used (environmentally relevant ones denoted with green asterisk*). The number of relevant Galápagos species sharing a taxonomic family with the study species are listed.

Study species	Harm effects	Polymer	Shape	Particle Size	Concentrations	Reference	Relevant Galápagos species
Ragworm *Hediste diversicolor*	**Cellular:** decreased coelomocyte viability but no change in phagocytosis	Polyethylene & polypropylene	Fragments	400 µm	10 mg kg^{-1} & 50 mg kg^{-1} in sediment, 10 & 100 µg L^{-1} in water	Revel *et al.*, 2018	**Nereidae:** 3 spp. *Ceratonereis monronis, Pseudonereis galapagensis, Pseudonereis variegata*
	Cellular: impaired functioning and genotoxicity in coelomocytes (immune effector cells) **Organism:** oxidative stress in tissues	PVC Benzo(a)Pyrene spiked PVC	Beads/ spheres	250 µm	200 & 2,000 particles kg^{-1}	Gomiero *et al.*, 2018	
	Organism: significant decreases in weight and feeding rate	Anti-fouling paint particles (acrylic with copper-based biocide)	Paint particles	100 µm – 1 mm	2.1 - 4.2 g L^{-1}	Muller-Karanossos *et al.*, 2020	
Polychaete *Perinereis aibuhitensis*	**Organism:** reduced regeneration rate **Population:** increased mortality rate	Polystyrene	Beads/ spheres	8 - 12 µm & 32 - 38 µm	100 and 1,000 particles mL^{-1}	Leung and Chan, 2018	
Scleractinian coral *Pocillopora damicornis*	**Sub-cellular:** transcriptome moderation **Organism:** repressed detoxification mechanisms and immune system	Polystyrene	Beads/ spheres	1 µm	50 mg L^{-1}, 9.0×10^{10} particles L^{-1}	Tang *et al.*, 2018	**Pocilloporidae:** 11 spp.
Common cockle *Cerastoderma edule*	**Population:** 100% mortality of all replicates before endpoints were measured.	Anti-fouling paint particles (acrylic with Cu-based biocide)	Paint particles	100 µm – 1 mm	2.1 - 4.2 g L^{-1}	Muller-Karanossos *et al.*, 2020	**Cardiidae:** 9 spp.
Blood cockle *Tegillarca granosa*	**Sub-cellular:** increased reactive oxygen species (ROS) in haemocytes **Cellular:** increased apoptosis **Organism:** suppressed immune responses, reduced phagocytosis	Polystyrene spiked with sertraline (anti-depressant)	Beads/ spheres	30 µm	0.29 mg L^{-1}*	Shi *et al.*, 2020	**Arcidae:** 18 spp. including the native reeve arc *Barbatia reeveana*
	Sub-cellular: increased ROS in haemocytes **Cellular:** increased apoptosis, decreased lysosomal membrane stability	Polystyrene & Benzo(a)Pyrene	Beads/ spheres	30 µm	1 mg L^{-1}	Tang *et al.*, 2020	

	Organism: immunotoxicity, reduced phagocytosis						
Thick shell mussel *Mytilus coruscus*	**Cellular:** slight impacts on oxidative responses with acidification, significant responses on digestive enzymes	Polystyrene	Spheres	2 µm	0, 10*, 10^4 and 10^6 particles L^{-1})	Wang *et al.*, 2020	**Mytilidae:** 21 spp. including the native sculptured mussel *Brachidontes puntarenensis*
Blue mussel *Mytilus edulis*	**Cellular:** decrease in lysosomal membrane stability **Organism:** inflammation	Polyethylene	Fragments	< 80 µm	2.5 g L^{-1}	von Moos *et al.*, 2012	
	Organism: decreased feeding rate	Polylactic acid & Polyethylene	Fragments	0.6 - 316 µm	2.5 & 25 µg L^{-1}	Green *et al.*, 2016	
	Cellular: increased antioxidant enzymes	Polyethylene & polypropylene	Fragments	< 400 µm	0.008 µg L^{-1}*, 10 µg L^{-1}, and 100 µg L^{-1}	Revel *et al.*, 2019	
Mediterranean mussel *Mytilus galloprovincialis*	**Cellular:** peroxisomal proliferation, neurotoxic effects, onset of genotoxicity **Organism:** localization in haemolymph, gills and digestive tissues, altered immunological responses	Polyethylene & polystyrene with polycylic aromatic hydrocarbons (PAHs)	Fragments	< 100 µm	1.5 g L^{-1}	Avio *et al.*, 2015	
	Subcellular: transcriptome modulation **Cellular:** apoptosis, stress protein response **Organism:** reduction in energy allocations (impaired growth)	Polyethylene	Beads/ spheres	1 – 50 µm	460,000 particles L^{-1}	Détrée & Gallardo-Escarate, 2018	
	Organism: inflammation response	Polystyrene	Beads/ spheres	4.5 µm	1,000 particles mL^{-1}	González-Soto *et al.*, 2019	
	Cellular: decrease in lysosomal membrane stability, significant immune response **Organism:** Transfer of Benzo(a)Pyrene to tissues	Polyethylene Benzo(a)Pyrene	Fragments	20 - 25 µm	10 mg L^{-1} $2.34 * 10^7$ particles L^{-1}	Pittura *et al.*, 2018	
	Cellular: changes in antioxidant enzymes	Phenol-formaldehyde plastic and “biofoam”	Foam fragments	170 ± 148 µm & 156 ± 56 µm	1 mg particles mL^{-1}	Trestrail *et al.*, 2020	
	Cellular: Increased reactive oxygen species production, reduced catalase activity	Polystyrene Fluoranthene	Spheres	2 and 6 µm	32 µg L^{-1}	Paul-Pont *et al.*, 2016	
Pacific oyster *Crassostrea gigas*	**Cellular:** increased size of immune cells including granulocytes **Population:** Significant reduction in reproductive success	Polystyrene	Beads/ spheres	2 and 6 µm	100 particles mL^{-1} 0.023 mg L^{-1}	Sussarellu *et al.*, 2016	**Ostreidae** 9 spp. (all < 4)

Clam *Scrobicularia plana*	**Cellular:** Some effects in biomarkers but mostly from Benzo(a)Pyrene **Organism:** Mechanical gill damage from microplastics	Polyethylene Benzo(a)Pyrene	Beads/ spheres	11 - 13 µm	1 mg L^{-1}	O'Donovan *et al.*, 2018	**Semelidae** 13 spp. including the endemic *Semele sowerbyi* (score 6)
Copepod *Calanus finmarchicus*	**Population:** premature moulting in juvenile copepods	Nylon	Fibres & Fragments	10− 30 µm	50 microplastics mL^{-1}	Cole *et al.*, 2019	**Calanidae** 12 spp.
Copepod *Calanus helgolandicus*	**Population:** impact on feeding and fecundity	Polystyrene	Beads/ spheres	20 µm	75 microplastics mL^{-1}	Cole *et al.*, 2015	**Calanidae** 12 spp.
Copepod *Parvocalanus crassirostris*	**Population:** substantial reduction in egg production	Polyethylene terephthalate (PET) & plasticizer (DEHP)	Fragments	5 - 10 µm	10,000–80,000 particles mL^{-1} DEHP (0.1–0.3 µg L^{-1})	Heindler *et al.*, 2017	**Paracalanidae** 2 spp. *Paracalanus denudatus, Paracalanus parvus,*
Brine shrimp *Artemia franciscana*	**Population:** reduced reproductive success	Polyethylene	Beads/ spheres	1–5 µm diameter	0.4, 0.8 & 1.6 mg L^{-1}	Peixoto *et al.*, 2019	**Artemiidae** 1 sp *Artemia salina*
Grass shrimp *Palaemonetes pugio*	**Population:** mortality (ranged 0% - 55%)	Polyethylene, Polypropylene, Polystyrene	Beads, Fibres and Fragments	30 - 165 µm	50,000 particles L^{-1}	Gray & Weinstein, 2017	**Palaemonidae** 21 spp. including the endemic shrimp *Palaemonella asymmetrica*
Common shore crab *Carcinus maenas*	**Organism:** transient impact on respiration following acute exposure	Polystyrene	Beads/ spheres	8 µm	10^6 - 10^7 particles L^{-1}	Watts *et al.*, 2016	**Portunidae** 8 spp. including the endemic swimming crabs *Achelous angustus* and *Achelous stanfordi*
Pacific mole crab *Emerita analoga*	**Population:** mortality increased, reduced reproductive success, disrupted embryonic development	Polypropylene	Fibres	1 mm	3 particles L^{-1}* added every 4 days	Horn *et al.*, 2020	**Hippidae** 4 spp. *Chaetodipterus zonatus, Emerita rathbunae, Emerita talpoida, Hippa pacifica*

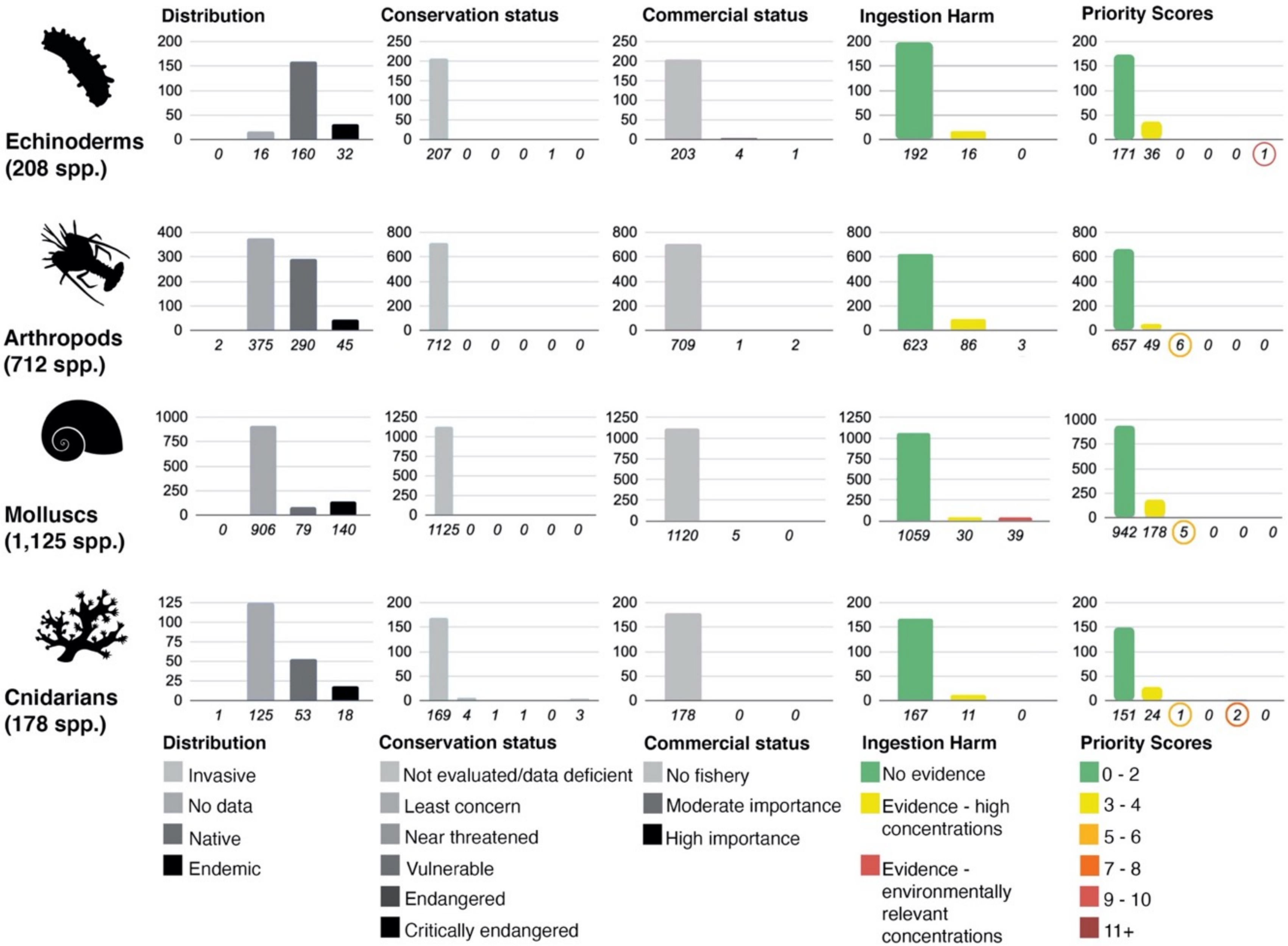


Figure 3.1: Prioritisation scoring of key marine invertebrate groups of Galápagos. The fifteen highest scoring species are denoted by circles in the final column (further described in Table 3.4).

Table 3.4: Table of the 15 Galápagos marine invertebrate species scoring S^P > 5 in the priority scoring analysis. Endemism status was determined using the Charles Darwin Foundation DataZone (https://www.darwinfoundation.org/en/datazone) and conservation status was retrieved from the IUCN Red List (https://www.iucnredlist.org/, retrieved 09/02/2021).

Top Scoring Species	**Species Distribution**	**Conservation Status**	**Commercial Importance**	**Example Evidence from the Literature**
Brown sea cucumber *Isosthichopus fuscus* S^P = 18	Native, found across the Eastern Tropical Pacific.	Endangered	Considered economically extinct due to severe overexploitation for the East Asian market since the 1990s (Toral-Granda *et al.*, 2008).	The uptake of microplastics in sea cucumbers has been reported in shallow reefs to the deep sea (Renzi *et al*., 2020; Taylor *et al.*, 2016). Laboratory exposure of four common species showed a selection preference for PVC fragments and nylon line over natural sediment, particularly those < 500 um in size (Graham & Thompson, 2009). No evidence of harm from microplastics was found.
Red spiny lobster *Panulirus penicillatus* S^P = 6	Native, circum-tropical range	Least Concern	Heavily exploited, primarily for export.	No literature evidence found for large lobsters.
Green spiny lobster *Panulirus gracilis* S^P = 6	Native, circum-tropical range	Data Deficient	Heavily exploited, primarily for export.	No literature evidence found for large lobsters.
Galápagos slipper lobster *Scyllarides astori* S^P = 6	Native to the Eastern Tropical Pacific, largest populations in Galápagos	Data Deficient	Moderately exploited, primarily for local consumption	No literature evidence found for large lobsters.
Asymmetric shrimp *Palaemonella asymmetrica* S^P = 6	Endemic to Galápagos	Critically Endangered	N/A	Increased mortality following exposure to high concentrations of different shaped particles of different polymer types in shrimps, with larger size fibres increasing harmful effects (Gray & Weinstein, 2017).
Swimming crabs *Achelous angustus* & *Achelous stanfordi* S^P = 6	Endemic to Galápagos	Not evaluated	N/A	Respiration impacted via gill obstruction in shore crabs (Watts *et al.*, 2016).
Galápagos giant chitons *Chiton goodalli* & *Chiton sulcatus* S^P = 6	Endemic to Galápagos	Not evaluated	Moderately exploited, primarily for local consumption	No literature evidence found for chitons.

Semelid clam *Semele sowerbyi* S^P = 6	Endemic to Galápagos	Not evaluated	N/A	Mechanical gill damage caused by microplastic exposure (O'Donovan *et al.*, 2018).
Sculptured mussel *Brachidontes puntarenensis* S^P = 6	Endemic to Galápagos	Not evaluated	N/A	Multiple impacts across ten studies (see Table 3).
Common reeve arc *Barbatia reeveana* S^P = 6	Native to Galápagos	Not evaluated	N/A	Increased reactive oxygen species (ROS) in haemocytes, increased apoptosis, suppressed immune responses, reduced phagocytosis (Shi *et al.*, 2020; Tang *et al.*, 2020).
Stony coral *Rhizopsammia wellingtoni* S^P = 9	Endemic to Galápagos	Critically endangered	N/A	Ingestion of microplastics has been linked with disturbance of the anthozoan-algae symbiotic relationship in laboratory simulations (Okubo *et al.*, 2018), also bleaching and tissue necrosis (Reichert *et al.*, 2018). Coral reefs are sparse in Galápagos following population crashes after the 1982 – 1983 El Niño event and have still not recovered in more acidic upwelling zones, highlighting major conservation priority (Manzello *et al.*, 2014).
Stony coral *Tubastraea floreana* S^P = 9	Endemic to Galápagos	Critically Endangered	N/A	
Caryophylliid coral *Polycyathus isabela* S^P = 9	Endemic to Galápagos	Vulnerable	N/A	

3.4. Discussion

The utility of a rapid risk assessment depends strongly on the quality of the evidence base on which it is constructed. Due to limited knowledge of marine invertebrate distributions, the lack of IUCN assessment, and the difficulty of characterising harm attributed to microplastic ingestion at environmentally relevant concentrations, only 15 species (0.6% of the total assessed) were highlighted for prioritisation from this scoring analysis (S^P > 5) compared to 69 vertebrates (9.7%) assessed in **Chapter 2**, a number that would likely be higher if the commercial scoring was applied to fish species. Nevertheless, this approach offers a simple tool to direct initial research attention to establish potential impacts to the base of the Galápagos marine food web in the absence of further data. Here the utility of the tool is discussed and some recommendations for future research directions are suggested.

The utility of this tool

This tool attempts to combine ecotoxicological and conservation risk assessment approaches. Due to the scoring mechanism, there is an inherent bias to species of higher conservation concern as they tend to be endemics, and in the case of the highest scoring species, the brown sea cucumber, its commercial exploitation has led to its IUCN assessment in the first place meaning these scoring categories are related (Hearn & Murillo, 2008; Toral-Granda *et al.*, 2008). Therefore, this tool is highlighting species that we would expect to be highest conservation priority, i.e. those we already know are threatened by other anthropogenic pressures (mostly over-fishing).

This study has highlighted the lack of ecotoxicological data for harmful impacts from microplastics in keystone invertebrate species such as sea cucumbers and lobsters. Sea cucumbers are generally deposit feeders having an important ecological role as detritivores and sediment modifiers (Toral-Granda *et al.*, 2008) although some species sometimes also employ suspension feeding strategies. They are also important dietary species for threatened vertebrates such as the endangered hawksbill turtle (*Eretmochelys imbricata*) (Fig. 3.2). Microplastic uptake has been described in wild and farmed sea cucumbers across the world (Mohsen *et al.*, 2019; Renzi *et al.*, 2020; Renzi & Blašković, 2020) and their likely value as 'passive samplers' of benthic sediments for microplastic contamination and other POPs due to their slow digestion processes has been proposed (Plee & Pomory, 2020; Renzi *et al.*, 2020). An exposure study with four sea cucumber species (including two *Holothuria* spp.) demonstrated selective uptake of nylon and PVC fragments that were recorded in the digestive tract of specimens at higher concentrations than expected considering the ratio of microplastic to sediment, perhaps due to the smooth particle surface enabling easier uptake (Graham & Thompson, 2009). Despite this documentation of uptake, no published evidence of harm was found in the literature suggesting a major knowledge gap around how sea cucumbers may be physiologically impacted by microplastics, suggesting further laboratory exposure studies are required to address this question of harm.

No evidence of uptake in large spiny lobsters or slipper lobsters was found in the literature, nor for adults of the American lobster (*Homarus* spp.) which is surprising considering their high economic importance across the Americas and Europe (Thunberg, 2007). As benthic invertebrates are the major diet for lobsters in Galápagos, the uptake measured in pencil urchins in **Chapter 2** demonstrate the potential of trophic transfer, warranting further investigation. One study was found in the literature concerning *Homarus americanus* larvae that showed differential effects across larval stages depending on the concentration of microplastics (polyethylene terephthalate fibres, mean size 500 μm, at a concentration gradient of 0, 1, 10 and 25 particles mL^{-1}) and the presence of food. The highest concentration affected survival only of early stage larvae and decreased oxygen consumption rates in older larvae (Woods *et al.*, 2020). Microplastic uptake by lobsters in Galápagos may be relatively easily studied by obtaining digestive tracts of fished lobsters as oftentimes the tails are the only part used and exported. Therefore, if sampled and stored using a robust protocol to control for contamination, addressing this knowledge gap would not necessarily require additional sacrifice of organisms.

The other higher scoring species were endemic and/or threatened species sharing a family with species used in exposure studies to date (bivalves, crustaceans and stony corals). Microplastic exposure studies are mostly based in Europe and thus, if the scoring criteria were adapted to consider feeding guilds or ecological niches as opposed to using taxonomic relationships, it may reveal more species of higher concern in Galápagos.

Figure 3.2: Hawksbill turtle (*Eretmochelys imbricata*) eating a brown sea cucumber (*Isosthichopus fuscus*) photographed in San Cristóbal Island, April 2019 (credit: Adam Porter).

Differences between priority scoring for vertebrates and invertebrates

The species-level approach used here may not be as useful for establishing potential risks in invertebrates as it is for vertebrates, coinciding with a recent review of microplastic ingestion in seafood species that showed uptake in bivalves did not tend to be species-specific (Walkinshaw *et al.*, 2020). To have the best chance of predicting population or ecosystem level effects, a traits-based approach might be most suitable. Traits-based analysis in ecotoxicology embraces ecological principles to scale up ecotoxicological knowledge gathered in the laboratory from a few species to apply findings at an ecosystem level to support risk assessment of the potential adverse effects of contaminants. Rubach *et al.* (2011) propose a framework including suggested traits based on external exposure (e.g. habitat use and food choice), intrinsic sensitivity (e.g. bioaccumulation, stress responses) and population sustainability (e.g. demography and recolonisation capabilities) (Rubach *et al.*, 2011). Traits-based meta-analyses considering fish and invertebrates have shown that microplastics do not tend to increase predictably with trophic level, in fact, lower trophic level species are both more likely to ingest microplastics due to crossover with dietary particle size ranges and may be more likely to suffer adverse health effects due to the greater surface area compared to the organism size (Covernton *et al.*, 2021; Walkinshaw *et al.*, 2020).

During the method development for this chapter, the definition of harm had to be adjusted for invertebrates due to the different evidence base available. In vertebrates, serious harm from plastic ingestion is much easier to observe, due to the fact that vertebrates are generally capable of consuming larger items that might cause complete blockage of the digestive system or injuries such as the perforation of organs (Alexiadou *et al.*, 2019; Brandão *et al.*, 2011). For the vertebrate scoring analysis, all laboratory exposures for fish where concentrations were above environmentally relevant concentrations were discounted. In many cases, for invertebrates it has been necessary to use high microplastic concentrations to garner measurable effects in exposures (Cole *et al.*, 2013) although as Koelmans *et al.* (2017) point out, ‘the realistic concentrations of today are not the realistic concentration of tomorrow’ highlighting that with increased plastic production and fragmentation, higher than present concentrations are likely to be relevant in many habitats (Koelmans *et al.*, 2017).

Evidence for vertebrate impacts in the field can be measured by non-expert members of the public as demonstrated through successful citizen science projects where entanglement incidences have been recorded, sometimes over decades by beach goers, divers and fishers (Donnelly-Greenan *et al.*, 2019; Parton *et al.*, 2019). Harmful interactions for invertebrates are harder to observe, due to the fact that impacts are primarily sub-lethal. To assess ingestion, organisms generally need to be sacrificed and processed in laboratory conditions meaning that public reports are unlikely to be possible. The plight of vertebrate individuals is generally much more emotive, as demonstrated by the '*Blue Planet II*' effect where a single sperm whale suggested to potentially have ingested plastics has been heralded as a major public motivation to address the plastic problem (Dunn *et al.*, 2020). For invertebrates, the focus is more often on effects at the population level, generally in the context of the knock-on effects on charismatic vertebrates.

What species might be missed by this tool and why?

Of the invertebrates considered in this study, 251 (10%) are endemic, however no distribution data is available for 1,500 (61%). In some taxonomic groups, further investigation (particularly with modern genetic tools) is likely to yield higher endemism, particularly in under-sampled habitats such as the deep-sea benthos, as recently demonstrated by the discovery of 30 new invertebrate taxa (including five genera new to science) after seven sites were explored within the Galápagos Marine Reserve > 3,000 m depth (Salinas-de-León *et al.*, 2020). In other taxonomic groups, further sampling efforts in the Eastern Tropical Pacific are likely to reduce the incidence of endemism, shown recently by observations of the Galápagos octopus (*Octopus oculifer*) for the first time in the Revillagigado Archipelago, Mexico (Valdez-Cibrián *et al.*, 2020) and the Galápagos slipper lobster in Cocos Island National Park, Costa Rica (Azofeifa-Solano *et al.*, 2016). These knowledge gaps in the current understanding of biogeographic distributions are the "Wallacean shortfall", where the cryptic nature of many species and the logistical challenges of sampling represent significant challenges to conservation and our understanding of the potential effects of future climate change (Brito, 2010; Whittaker *et al.*, 2005).

The majority of Galápagos marine invertebrates have never been assessed by IUCN (2,431 spp., 99%) meaning that there is a high chance we don't actually know how many species are threatened. Only four species (< 0.01%) are considered threatened (i.e. vulnerable, endangered or critically endangered according to the IUCN Red List), namely the brown sea cucumber (*I. fuscus*) and three endemic stony coral species including the critically endangered Wellington's solitary coral (*Rhizopsammia wellingtoni*) and sun cup coral (*Tubastraea floreana*) and a vulnerable colonial coral (*Polycyathus Isabela*). Simultaneously, only four species are listed as of 'Least Concern' following assessment including the red spiny lobster (*Panulirus penicillatus*) due to its circum-tropical distribution and three cosmopolitan stony corals (*Pavona clavus*, *Psammocora stellata*, *Fungia distorta*). Eight species are categorised as Data Deficient including one stony coral (*Antipathes galapagensis*), the Galápagos abalone (*Haliotis dalli*), the slipper lobster (*Scyllarides astori*), and the warty sea cucumber (*Stichopus horrens*) among others. These were scored as low conservation priority in this analysis but perhaps should be scored higher if a precautionary approach is taken, due to the fact that they were of high enough concern for an assessment to be attempted in the first place.

Data requirements for IUCN assessment are extensive and expensive, requiring population data trends over ten years or three generations, well-defined range sizes and knowledge of threat responses for quantitative models used to estimate extinction risk (Rodrigues *et al.*, 2006). From the biogeographic perspective, the most threatened, endemic marine invertebrates with restricted ranges are situated in the Western Zone of the Galápagos Marine Reserve (Edgar *et al.*, 2008) suggesting this area could be the major focus of attention. This should be mapped against the areas of highest plastic contamination to highlight key hotspots where exposure is most likely, as done recently for the Mediterranean marine food web (Compa *et al.*, 2019). Although we can't be sure of the impacts of microplastics on the

Galápagos marine food web at this early stage of investigation, this information will form important baseline data for wider conservation application.

What other invertebrate:plastic interactions exist?

This scoring analysis focused solely on ingestion of microplastics, however a wider range of invertebrate:plastic interactions have been described that deserve further attention. I did not score invertebrates for prioritisation in terms of entanglement due to the scarcity of published information. Analogies have been made between entanglement and the smothering of reef-building corals, where snagged plastic debris increases disease risk and has been linked with reef die offs (Lamb *et al.*, 2018). During our field campaign in May 2018, we observed a Galápagos green sea urchin (*Lytechinus semituberculatus*) covered in yellow plastic tape (Fig. 3.3), emulating typical behaviour of covering themselves with macroalgae to camouflage in the environment, in this case having presumably the opposite effect of affording enhanced protection.

Plastics have been shown to affect larval settlement dynamics with impacts for rafting communities and the potential introduction of invasive species. A study by Pinochet *et al.*, (2020) showed an active preference of invasive bryozoans (*Bugula neritina* and *Bugula flabellata*) for settling on plastics as opposed to other materials such as concrete and wood. Faster settlement saved energy resulting in increasing individual fitness coupled with the propensity of plastics to float large distances, meaning that distribution ranges are likely to be increased as a function of more plastic in the environment (Pinochet *et al.*, 2020).

Figure 3.3: Galápagos green sea urchin (*Lytechinus semituberculatus*) covered in yellow plastic tape photographed in San Cristóbal Island, May 2018 (credit: Adam Porter).

Eisenhauer *et al.* (2019) recommend a threefold approach to filling major knowledge gaps for invertebrate biodiversity trends that might be embraced for Galápagos; (i) the mobilisation of existing data coupled with innovative statistical techniques to allow for the compilation of datasets spanning different spatiotemporal resolution, (ii) targeted survey in well-sampled sites to elucidate longer term trends and (iii) national scale monitoring, supported by strengthened capacity in taxonomic analysis (Eisenhauer *et al.*, 2019). Combined with laboratory exposures to better describe harm at various biological levels, ideally using particles and concentrations more reflective of environmental exposure will support the better prediction of harm effects. The combination of ecotoxicological information as well as physiological mechanisms, such as ingestion and egestion rates, overlaid with an increasing resolution of environmental distribution information will support the construction of ecotoxicological models such as Ecotracer to map potential impacts at different trophic levels and support predictions of future effects.

3.5. Conclusion

Priority scoring analysis presents a rapid assessment approach that has proved to be useful for highlighting some priority Galápagos marine invertebrates for research attention and mitigation. However, it is limited by incomplete data for most of the scoring parameters suggesting that in the interim, a more precautionary approach to assessing risk should be employed due to the vulnerability of this ecosystem. To address the data gaps required, perhaps a traits-based analysis would compensate for the lack of sampling of marine invertebrates in the Galápagos Marine Reserve and across the Eastern Tropical Pacific region to date. Genetic tools may also fill some knowledge gaps for populations that can be paired with threat analysis using ecotoxicological techniques and ecological modelling to assess conservation status, embracing a multi-disciplinary approach. Overlaid with better spatiotemporal data of environmental plastic contamination, we will better be able to define health risks to build knowledge and our capacity to predict future impacts in the face of global change. The actions required to address these data gaps are likely to have wider conservation benefits for wider marine biodiversity due to the ecological importance of marine invertebrates and the need for better tools for resource management.

Chapter 4

Characterisation of plastic contamination and its association with marine macroalgae across two biogeographic zones in the Galápagos Archipelago

Abstract

The biogeographic zones of the Galápagos Marine Reserve are highly distinctive in terms of the influence of oceanographic currents, environmental conditions, ecology and human usage, hence may also be differentially influenced by plastics. Here, the distribution and composition of plastic contamination across marine habitats were compared between two biogeographic zones; (i) the Western Zone (where oceanographic models predict floating plastics would be transported away from the Archipelago due to the action of upwelling and offshore currents) and (ii) the South-Central Zone (where human activity is elevated and the incoming Humboldt Current increases connectivity with the continent), to test the hypothesis that contamination would be higher in the latter. Environmental macroplastic and microplastic concentrations were measured via beach survey, sea surface trawls and benthic sediment sampling and algae:microplastic association was described using a common species of green macroalgae (*Ulva lobata*) growing on intertidal rocks found in both zones. Microplastic concentrations at the seawater surface, in sand and in benthic sediment were significantly higher (ten-fold (p = 0.002), six-fold (p = 0.02) and eight-fold (p = 0.03) respectively) in the populated South-Central Zone. Significant differences were not found between biogeographic zones for abundance of macroplastic or sieved beach microplastics (1 – 5 mm). There was no clear pattern for algae:macroplastic association, attributed to high variability between sites and the (positive) impact on reduction of larger plastic debris from cleaning activities in the South-Central Zone. Microplastics were found associated with 36.8% of *U. lobata* fronds (n = 21) collected from

across both zones with a mean density of 0.35 particles per gram wet weight, twice the concentration reported recently in Chinese *Ulva* sp. mariculture farms. Algae does not appear to present a viable bioindicator for environmental microplastic contamination as concentrations did not reflect contamination of any habitat compartment. Overall, these results supported the original hypothesis that plastic contamination is generally lower in the Western Zone and demonstrate that macroalgae presents a potential entry pathway for microplastics into the Galápagos marine food web.

4.1. Introduction

Biogeography is defined as the geographic distribution of species, generally influenced by predictable environmental variation such as temperature and oxygen gradients and the abilities of different species to adapt to them (Deutsch *et al.*, 2020; Lomolino, 2000). In the marine environment, dynamic environmental variation and latitudinal differences in winds, currents and geology interact with ecology to create biogeographic zones that can be defined at a variety of scales (Hedgpath & Ladd, 1957). Many of these environmental variables are also linked to the movement and fate of plastics in the marine environment (Galloway *et al.*, 2017; Zhang, 2017), although studies at regional scales large enough to consider differences between biogeographic zones are lacking in the literature. Biogeographic origins of plastics have been traced using microbial and biofouling communities on their surfaces (Amaral-Zettler *et al.*, 2015) but little evidence exists for the effects of plastic contamination on different biogeographic communities, nor for the effects of different communities on modulating plastic movements, for example, the enhanced fouling of plastic in higher biomass regions or the temporary sequestration of plastics following incorporation into the food web.

At a global scale, the high incidence of species endemism in the Galápagos Marine Reserve (18.2% of described marine species) qualifies this region as the only tropical archipelago to meet the criteria of a biogeographic province. All other islands in the Eastern Tropical Pacific are considered outposts of the Panamanian province (Briggs & Bowen, 2012). Within the boundary of the Galápagos Marine Reserve, environmental conditions vary substantially due to the differential impact of oceanic currents and the strong seasonal upwelling of the Equatorial Undercurrent to the west (Palacios, 2004). This causes high nutrient input from the vertical transport of deep waters that drives high primary production, particularly in the coolest months (August – October) (Palacios, 2004; Schaeffer *et al.*, 2008). These conditions have influenced species distributions including macroalgae, reef fish and macroinvertebrate communities and thus resulted in three major biogeographic zones and two 'sub-zones' in the Galápagos Marine Reserve, as defined by Edgar *et al.* (2004). These are: i) the temperate and cold **Western Zone** (sometimes sub-divided into the additional endemism hotspot of the **Elizabeth Zone** between Fernandina and Isabela islands), ii) the tropical and warm **Northern Zone** (sometimes sub-divided to differentiate the **Far-Northern Zone** around Darwin and Wolf islands) and iii) the sub-tropical, mixed **South-Central Zone** which also hosts all human residents and the bulk of tourism activity (Edgar *et al.*, 2004b) (see Fig. 4.1). Table 4.1 further characterises these zones describing key species and the profile of human usage.

Macroalgae are important for global marine biodiversity, providing habitat and food for diverse biological communities and playing a major role in biogeochemical cycling (Duffy *et al.*, 2019). The high diversity of macroalgae in Galápagos is more typical of temperate ecosystems than the equatorial tropics with > 300 species described (36% endemic), dating back to Charles Darwin's HMS *Beagle* records (James, 1991; Tompkins & Wolff, 2016). Elevated nutrient availability means macroalgal communities in the Western Zone are larger, more diverse and more speciose and support dynamic communities of herbivores including the largest colonies of the endemic marine iguana (*Amblyrhynchus cristatus*). Macroalgae are important in the South-Central Zone but are more patchily distributed and are virtually absent in the warmer, nutrient-poor Northern Zone and thus this region is not included in this study (Tompkins & Wolff, 2016; Vinueza *et al.*, 2014).

The role of marine vegetation including macroalgae, seagrasses and mangroves in modifying plastic pathways and fates in coastal environments has received little research attention to date (perhaps with the exception of mangroves), despite the potential to substantially affect accumulation (Feng *et al.*, 2020; Yokota *et al.*, 2017). At the macro-scale, the branching and root structures of marine vegetation can cause larger plastic debris to snag and accumulate (particularly fibrous and film-like items), presenting an entanglement risk, including for shorebirds (Ivar Do Sul & Costa, 2014). Vegetation may provide a physical barrier causing the retention of plastics, as seen in mangrove forests where plastic concentration is directly related to tree density (Martin *et al.*, 2019). This may increase the *in situ* generation of microplastics that may be more likely to accumulate in sediments due to particulate matter being trapped in root systems (Cozzolino *et al.*, 2020; Govender *et al.*, 2020). Microplastics can attach to vegetation surfaces, as reported on seagrass (*Thalassia testudinum*) in the Western Atlantic (Goss *et al.*, 2018) and on rafting macroalgae (*Ulva* spp.) mats in the Yellow Sea (Feng *et al.*, 2020). Several mechanisms for attachment have been proposed including wrapping around algal fronds and holdfasts, embedment in the plant tissue, epibionts (defined as an organism living on the surface of another) 'catching' plastics and sequestering them onto the plant's surface, and also the adhesive effect of biofilms as a sticky trap (Feng *et al.*, 2020; Goss *et al.*, 2018). Macroalgae have been proposed as a more reliable bioindicator for environmental microplastic contamination than animal species as it is static, although this has not yet been extensively tested (Feng *et al.*, 2020).

Table 4.1: Characteristics of the marine biogeographic zones of the Galápagos Marine Reserve (after Edgar *et al.* 2004; Tompkins and Wolff 2016).

Zone	Ecological Profile	Human Use
Western Zone (temperate/ cold)	• High inter-site variability compared to other zones • Highest primary productivity • High proportion of 'Peruvian' cold water species • Large, diverse and speciose macroalgal assemblages • High numbers of endemic fish species • With the Elizabeth Zone, hosts the majority of the endemic flightless cormorant (*Phalacrocorax harrisi*), Galápagos penguin (*Spheniscus mendiculus*) and Galápagos fur seal (*Arctocephalus galapagoensis*) populations, all scored at high risk of negative interactions upon encounter of plastics (see **Chapter 2**) • Home to the largest colonies of marine iguanas (*Amblyrhynchus cristatus*)	• No human population • Small number of tightly managed visitor sites open only to scheduled tour groups from cruise boats landing for < 2 hours in groups of 16 accompanied by a Naturalist Guide • Seasonal fishing
Elizabeth Zone (temperate/ cold)	• Highest species endemism • Highest primary productivity • Large, diverse and speciose macroalgal assemblages • Highest density of commercially valuable groupers (*Paralabrax albomaculatus* and *Mycteroperca olfax*) • Highest density of warty sea cucumber (*Stichopus fuscus*)	• No human population • Limited tourism • Seasonal fishing
Northern Zone (tropical/ warm)	• Considered virtually barren in terms of macroalgae • Most developed coral reefs • Mixture of species from the South-Central Zone and Far-Northern Zone	• No human population • Small number of visitor sites open only to scheduled tour groups from cruise boats landing for < 2 hours • Some fishing
Far-Northern Zone (tropical/ warm)	• Considered virtually barren in terms of macroalgae • Most developed coral reefs • High reef fish species richness, high proportion of Indo-Pacific species • Highest fish biomass including large aggregations of scalloped hammerhead sharks (*Sphyrna lewini*) • Low species richness of mobile macro-invertebrates	• No human population • No land-based tourists, only dive tour groups visit this zone • No Take Zone proposed in 2016 • Illegal fishing is a major concern
South-Central Zone (sub-tropical/ mixed temperature)	• Species from a variety of sources including many of Panamanian origin • Patchily distributed macroalgae with high localised importance to communities • Some coral reefs on eastern coastlines although widescale bleaching and destruction occurred following strong El Niño events (particularly in 1983) • Home to the most endangered marine iguana subpopulation in Punta Pitt, San Cristóbal island (*Amblyrhynchus cristatus godzilla*) • Home to the largest colony of Galápagos sea lions in San Cristóbal (*Zalophus wollebaeki*)	• Home to all human residents (approx. 26,000) and the majority of tourists • High fishing activity

When considering the potential ecological impacts of contamination in a highly variable system such as Galápagos, biogeography must be considered as well as environmental factors and the presence of other anthropogenic pressures. In **Chapter 2**, I describe plastic contamination at an island-scale using the case study of San Cristóbal in the far-east of the South-Central Zone. Concurring with oceanographic models, higher accumulations of plastics were measured on the exposed wind-ward coastline, hypothesised to be due to the greater influence of the Humboldt Current. As yet, there are no published data on the concentrations of plastic contamination in the Western Zone of the Galápagos Marine Reserve. Anecdotal reports suggest that there is less plastic accumulating in the west, potentially explained by a smaller human presence and due to the strong upwelling and offshore currents that are likely to transport floating plastics away from island coastlines (Van Sebille *et al.*, 2019). This suggests that marine life in the Western Zone of the Archipelago may be less exposed to plastics and therefore less likely to be at risk of negative effects.

Here I investigate differences in the distribution and composition of macroplastic and microplastic across two biogeographic zones to test the hypothesis that plastic contamination is lower in the Western Zone than the South-Central Zone. Environmental plastic contamination was measured across habitats using beach surveys, sea surface trawls and benthic sediment sampling. Green algae were sampled to investigate the association of microplastics with marine vegetation and how this may vary with different environmental concentrations, selecting *Ulva lobata* as an important species for the rocky shore grazing community (Vinueza *et al.*, 2014) as well as representing a cosmopolitan genus with relevance for global marine ecosystems.

4.2. Methods

Study area and sampling design

Two groups of study sites were considered: Sites 1 – 4 located in the Western Zone on Fernandina and Isabela islands and Sites 5 – 9 located in the South-Central Zone on Floreana and San Cristóbal islands (Fig. 4.1). Each site was visited once between 21 – 28 August 2019, during the cool season when the effect of the Humboldt Current is strongest in the South-Central Zone and the upwelling is at its strongest in the West. A small local fishing vessel was hired as a live-aboard for sampling, shared with two other research groups. Each of the nine sampling sites was associated with a particular colony of marine iguana sub-species that were sampled by a collaborator for various health indicators including faecal samples to investigate the presence of microplastics.

Beaches varied considerably, particularly in size and sediment type; varying from fine sand to lava rubble boulders of approximately 4 – 8 cm. Due to logistical issues (limited boat time), Site 7 and Site 9 could not be sampled for benthic sediment nor for seawater surface concentrations and so data collected using the same methodologies in May 2018 were used as a proxy. Due to beach sediment type (boulders), Site 3 was not surveyed for beach microplastic as sieving was not possible (see **Chapter 6** for more information on this site). *Ulva lobata* was found growing accessibly in all sites apart from Site 4 where no samples were taken.

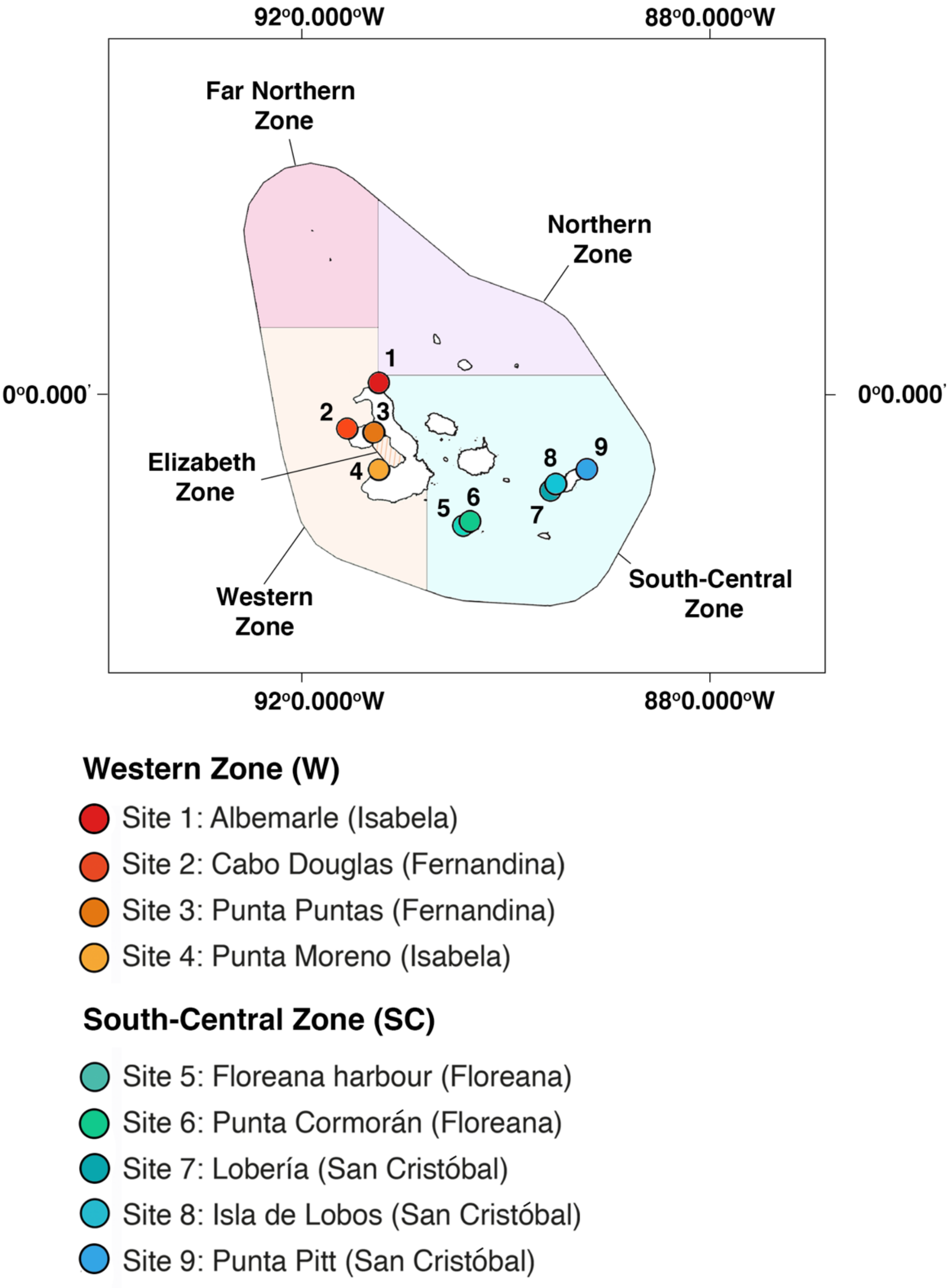


Figure 4.1: Map of the Galápagos Marine Reserve with numbered sampling sites and major biogeographic zones as defined by Edgar *et al.* (2004). Shapefiles for GIS provided by Moity (2019).

Environmental sampling and processing

Beach surveys

Two 50 m macroplastic transects were sampled at each side of each beach to generate representative data. For beaches smaller than 50 m, the whole beach was surveyed. All visible plastic items and fragments (> 5 mm) between the waterline and vegetation line (or raised lava line at some sites) were collected and later laid out for imaging with a tape measure for scale. Macroplastic was counted and categorised using the photographs using a modified OSPAR protocol refined in **Chapter 2** (OSPAR, 2010; Watts *et al.*, 2017), by myself, university students and several trained volunteers who were provided with an identification protocol and a reference list of categories (see **Chapter 2** Supplementary Table 2.2). This crowd-sourced data was spot-checked (20% items per site) to ensure consistency in categorisation and was deemed to be > 95% accurate. The most commonly misidentified items were tarinas (food take away pots that are common in Ecuador) and films/ sheets misidentified as plastic carrier bags. All photographs were checked for these items to ensure consistency in the remaining data. Beach area was calculated using satellite images (retrieved from Google Earth, June 2020) to convert data into items per m^2. A sub-sample of macroplastic items from each location was taken for Fourier Transform Infrared spectroscopy (FTIR) analysis to test for polymer similarity to smaller particle contamination (n = 140, approx. 10% of total sample).

Large microplastics (1 – 5 mm) were collected by sieving the top 50 mm of sand from three 50 cm x 50 cm x 50 mm quadrats, along each macroplastic transect, at least 5 m apart (total of six quadrats for most sites) following the methodology outlined in **Chapter 2** and **Chapter 5**. To sample the smaller size fractions of beach plastic (counts including all particles < 5 mm as opposed to having a 1 mm lower limit), triplicate 50 mL sand ‘cores’ were collected using centrifuge tubes at the strandline within the macroplastic transects, next to the sieved quadrats and were processed with the identical method as reported in **Chapter 2**, i.e. the density floatation protocol outlined by Coppock *et al.* (2017).

Sea surface

Seawater surface tows of 10 minutes at 2 knots boat speed were undertaken in triplicate using a 200 μm plankton net with a flow meter, towing into the wind, away from the shoreline starting approx. 20 m offshore, again, as per the method used in **Chapter 2**. Global Positioning System (GPS) readings were taken using a handheld Garmin Etrex 10 device at the start and end of each tow. Large floating macroalgae clumps (e.g. *Sargassum* spp.) were removed from the tow net during sampling to avoid clogging the net and influencing results due to any plastic accumulated on floating algae. However some samples, particularly those from the Western Zone, retained a substantial algae fraction. Samples were fixed in pre-filtered 4% formaldehyde solution in 500 mL Nalgene bottles. Nylon mesh (50 μm) was used for filtering, washed thoroughly in sterile laboratory conditions (submerged and agitated in three sequential beakers of Milli-Q water filtered to 0.22 μm). Ten filters were inspected using an Olympus MVX10 microscope for contamination confirming that washing was effective in removal of any potential fibrous microplastic contamination (no contamination was observed).

Additional processing steps were introduced for seawater samples compared to **Chapter 2** due to the generally higher fraction of vegetation in samples. Firstly, the formaldehyde solution was poured off carefully through a nylon mesh, leaving the solid matter in the sample bottle. This mesh was retained for inspection in a sealed petri dish. The sample bottles were then filled with Milli-Q water and were briefly submerged in an Ultrasonic bath for 60 seconds to remove any microplastics from algal matter. The sample was poured through a 5 mm mesh laboratory grade sieve with a collection tray to trap the majority of vegetation. Anything caught in the sieve was washed thoroughly with Milli-Q and the retained solution was filtered through a new 50 μm nylon mesh. Any remaining solid matter (primarily zooplankton) was returned to the original sample bottle and approximately 100 mL of 20% filtered potassium hydroxide (KOH) solution was added. Bottles were shaken vigorously and heated at 40°C for 48 hours with the lids covering the sample but not screwed on. Samples were shaken after addition of KOH, after 24 hours and after 48 hours. Samples were 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.

Benthic sediment

Benthic sediment samples were collected in triplicate at each location taking a 50 mL sample from a 250 cm^3 Van Veen Grab at 1 – 3 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.

Macroalgae sampling and processing

Triplicate samples of *Ulva lobata* attached to intertidal rocks were collected from all sites next to marine iguana colonies in foraging areas. Whole fronds (approx. 2 – 4 cm, mean wet weight 4 g, mean surface area 10 cm^2) were scraped from rock surfaces with a metal scalpel and stored in precleaned 40 mL borosilicate glass vials before freezing. In the laboratory, samples were defrosted in their glass bottles. In a fumehood, samples were transferred to pre-weighed petri dishes with forceps and immediately covered. Vials were rinsed three times with Milli-Q to remove any particles sticking to the glass, and the sample was vacuum-filtered onto a 10 μm polycarbonate filter. A wet weight was taken of the algae in the petri dishes using a microbalance (determined as the most useful unit due to historical herbivore diet records and other macroalgae studies reporting wet or 'fresh' weight (Wikelski *et al.*, 1993; Feng *et al.*, 2020)). The area of the fronds were visually estimated by unfurling samples. As per the method described by Feng *et al.* (2020), samples were transferred to glass conical flasks and digested in approximately 100 mL of Fenton's Reagent containing 30% (v/v) H_2O_2 and catalyst solution (20 g of $FeSO_4 \cdot 7H_2O$ in 1 L Milli-Q water) and covered with aluminium foil (Feng *et al.*, 2020). They were left in an oscillating incubator at 40°C for 24 hours before being vacuum-filtered through a 10 μm polycarbonate filter. This digestion step allows for more direct comparison with different species in the future compared to relying on microscopy that may be hindered by morphological differences of specimens affecting the ability to observe particles.

Polymer verification

Filters from environmental and algae samples were systematically examined using an Olympus MVX10 microscope and suspected synthetic particles were identified following the same visual criteria as in **Chapter 2**. Suspected synthetic particles were isolated, imaged, counted and categorized according to shape (fibre, fragment, foam, film, pellet) and colour. Particle numbers are likely to be biased towards colourful or dark particles that stand out from a background of digested plankton or algae matrix. It is therefore likely that colourless and white particles are undercounted in samples, particularly those < 1 mm in size. All suspected microplastic particles were analysed by FTIR spectroscopy. Some particles were too small to be picked out of the sample (< 100 μm, n = 23) or were lost during processing stages (n = 34). Particles were analysed using a PerkinElmer Frontier FTIR spectrometer using the attenuated total reflection (-ATR) universal diamond attachment for particles > 1 mm or a PerkinElmer Spotlight 400 μFTIR Imaging System 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 4,000 to 650 cm^{-1}). Linear normalisation and base-line correction tools from the Perkin-Elmer's Spectrum™ 10 software (version 10.5.4.738) were used to further refine spectra. A general threshold of 70% library match for FTIR polymer analysis was used, along with a strong visual spectra match to improve confidence in results.

Contamination control

Atmospheric controls (dampened glass fibre filters in an open petri dish) were collected in the field during water and sediment sampling to control for boat-borne and airborne contamination. For seawater tows, a dampened filter was held open during the entirety of the tow, held over the side of the boat facing the wind. Atmospheric controls were applied at all processing stages, by way of a dampened glass-fibre filter in a small petri dish. Procedural blanks were carried out during density separation and digestion stages of sample processing in the laboratory. A cotton lab coat and latex gloves were worn, and all open sample time was under a fumehood and limited as much as possible. All equipment and tools were rinsed three times with Milli-Q between each sample. Each of the procedural and atmospheric blanks underwent the same processing steps as samples. Contamination was extremely low, only one atmospheric blank (total = 15) had contamination; two blue cellulosic fibres during processing of beach sediment samples. Data were adjusted by removing two blue cellulosic fibres from each sample counted during the time that blank was in use. No microplastic contamination was recorded in the field blanks.

Data Analysis

All data were tested for normality using histograms and the Shapiro-Wilk test and did not meet the assumptions required for parametric statistics i.e. no datasets were normally distributed due to high variability. Differences between the Western and South-Central Zones were therefore tested using unpaired, two-sample Wilcoxon's tests and differences between multiple sites were tested using Kruskall-Wallis tests with Dunn's post-hoc test. Due to overdispersion of data, a negative binomial generalised linear model (GLM) was developed for each habitat compartment (beach macroplastic, large sieved microplastic, beach microplastic, seawater and benthic) following the same method as in **Chapter 2** but with the additional explanatory variables of island and biogeographic zone. Differences in the percentage composition of macroplastic items between biogeographic zones were tested with a Contingency Table Chi-Square Test. Pearson residuals were calculated to identify the items driving differences between zones. A correlation plot using Pearson's Correlation Coefficient was generated to test relationships between plastic abundance across different habitat compartments and algae:plastic association. Data analysis was undertaken in R Studio (Version 1.3.1073) and Microsoft Excel.

4.3. Results

Spatial distribution of plastic

When averaged over the beach area (items m^{-2}), macroplastic was 35% higher in the South-Central Zone than the Western Zone, although this was not significantly different (0.08 ± 0.05 (standard error) items m^{-2} vs 0.05 ± 0.03, Wilcoxon's Test, W = 11, p = 0.9; Fig. 4.2a). High variation was observed between sites with the highest accumulation of beach macroplastic at the east facing site of Punta Cormorán (Site 6, South-Central Zone) (0.25 items m^{-2}, 8.31 items m^{-1}) but the second highest at the most westward site (west facing) Cabo Douglas (Site 2, Western Zone) (0.12 items m^{-2}, 2.17 items m^{-1}). Large microplastic (1 – 5 mm) sieved from the top 50 mm of beach sediment was also higher in the South-Central Zone than the Western Zone but due to high variability, the difference was not significant (107.5 ± 51.5 particles m^{-2} vs. 12.6 ± 6.9 particles m^{-2}, Wilcoxon W = 181.5, p = 0.4, Fig. 4.2b). Anomalously high concentrations were collected from Punta Pitt (Site 9) (mean 355.3 ± 174.5 particles m^{-2}), defining this east facing beach in eastern San Cristóbal as an accumulation hotspot for large microplastics (as confirmed in **Chapter 2** and **Chapter 5**). The cleanest beach was Isla de Lobos (Site 8, South-Central Zone) despite its close proximity to the harbour of Puerto Baquerizo Moreno (7 km to the west) where no plastics > 1 mm were found at all.

Microplastic was significantly higher in the South-Central Zone compared to the Western Zone in beach sand (1.3 ± 0.6 particles per 50 g dry weight vs. 0.2 ±0.1 particles per 50 g dw, Wilcoxon's rank sum test, W = 133, p = 0.02, Fig. 4.2c), at the seawater surface (0.37 ± 0.13 particles m^{-3} vs. 0.03 ± 0.01 particles m^{-3}, W = 155, p = 0.002, Fig. 4.2d) and in benthic sediment (2.5 ± 1.1 particles per 50 g dw vs. 0.3 ± 0.3 particles per 50 g dw, W = 129.5, p = 0.03, Fig. 4.2e). There were significant differences between sites for seawater surface concentrations with the highest around Isla Lobos (Site 8) (1.23 particles m^{-3}, Kruskall Wallis test with Dunn's post hoc, H = 17.183, df = 8, p = 0.03) and for benthic concentrations that were highest at Punta Cormorán (Site 6) (6.7 particles 50 g dw, Kruskall Wallis test H = 19.1, df= 8, p = 0.01). GLMs for all plastic counts across habitats, using explanatory variables including biogeographic zone, island, beach aspect, windward/leeward orientation, site usage (tourist/ remote) and distance from port found no statistically significant drivers with the exception of large microplastics where beach aspect (driven by higher concentrations on east facing bays) (p < 0.001) and distance from port (p < 0.001) were significant drivers for plastic abundance (see Table 4.2). This is likely due to the anomalously high concentrations at Punta Pitt (Site 9) however.

Table 4.2: Summary results of best-fit negative binomial generalised linear models (GLMs) for environmental data. Explanatory variables explored included biogeographic zone, beach aspect (north, west, south, east), distance from port, grain size, windward vs leeward orientation and site usage. 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	-1.790	1.708	-1.048	0.295	15.485	0.705
	Beach aspect (West)	-2.349	5.314	-0.442	0.658		
Sieved microplastic (particles 1 – 5 mm)	Intercept	4.523	0.115	39.44	<0.001	101.36	29.242
	Beach aspect (East)	-1.731	0.199	-8.88	**<0.001***		
	Distance from port	0.021	0.002	10.27	**<0.001***		
Whole sand (particles < 5 mm)	Intercept	0.975	0.434	2.244	0.025	24.816	1.651
	Beach aspect (West)	-2.073	1.090	-1.902	0.057		
Seawater (particles < 5 mm)	Intercept	-0.703	0.805	-0.874	0.382	13.725	0.669
	Distance from port	-0.022	0.023	-0.949	0.343		
Benthic (particles < 5 mm)	Intercept	4.281	0.439	9.761	<0.001	33.576	1.385
	Distance from port	-0.012	0.008	-1.414	0.157		

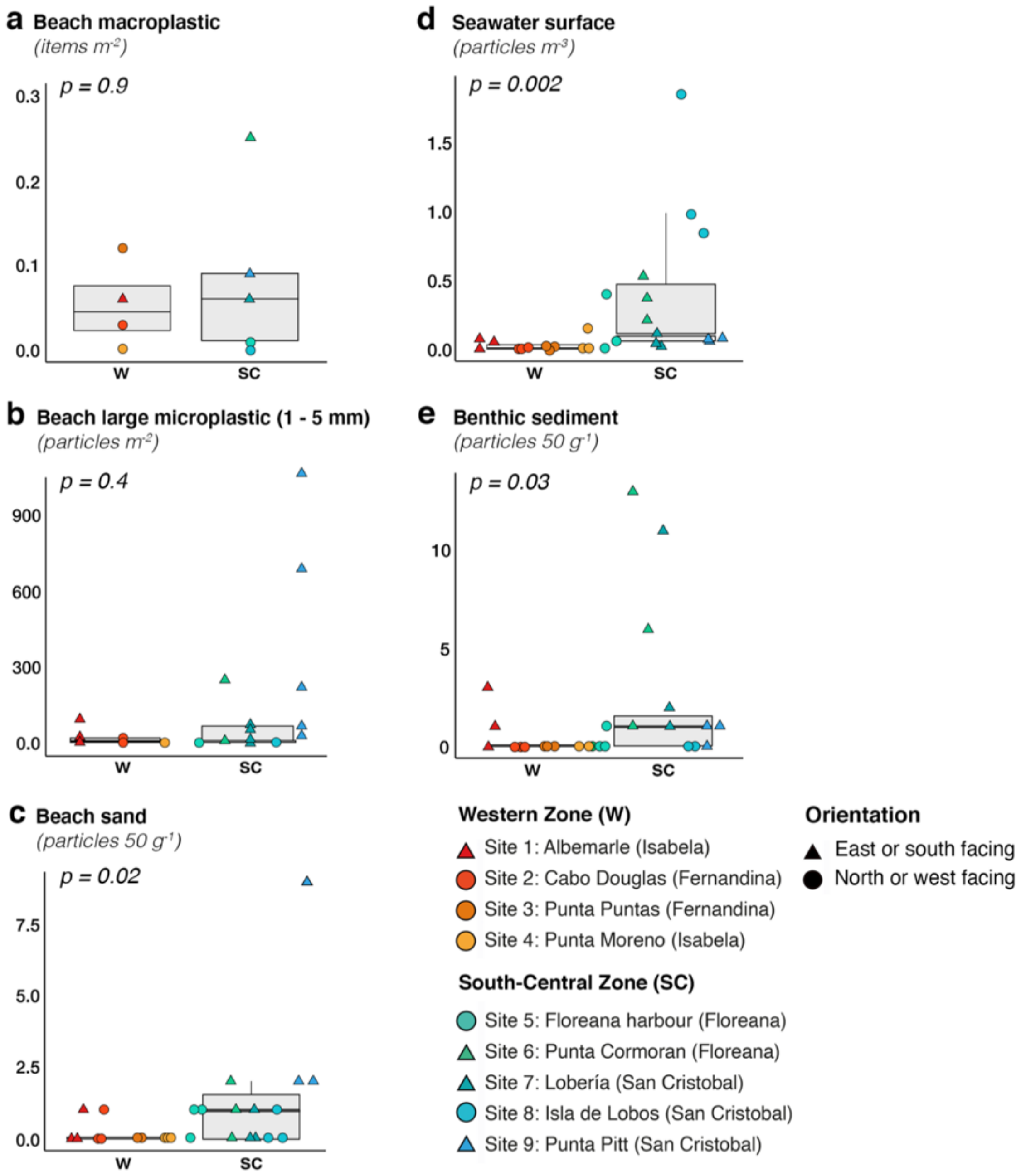


Figure 4.2: Plastic abundance across habitat compartments grouped by biogeographic zone (Western/ South-Central). (a) Beach macroplastic (items > 5 mm), (b) beach large microplastic sieved from the surface 50 mm (particles 1 – 5 mm), (c) beach sand (particles < 5 mm), (d) seawater surface (particles < 5 mm) and (e) benthic sediment (particles < 5 mm). Circles denote north or west facing sites and triangles denote east or south facing sites. Bold lines on box plots denote mean. *P* values from Wilcoxon Tests are included describing differences between means between zones.

Item composition of beach macroplastic

A total of 1,395 macroplastic items (including fragments > 5 mm) were collected, imaged and categorised across the sites. At the Archipelago scale, macroplastic was mostly unsourced hard fragments (66%), maritime items (16%, mostly ropes) or drinks packaging (10% including bottles, bottle caps and bottle rings). Although the relative percentage of fragments, ropes and bottle caps were even across the Archipelago (albeit at different densities), the overall composition of macroplastic varied significantly between zones (Chi-square Test; $\chi^2 = 25.42$, df = 9, $p = 0.002$) (Fig. 4.3). The drivers for these differences were the high presence of films and plastic sheeting in the Western Zone and the high incidence of drinks bottles and buoy fragments in the South-Central Zone.

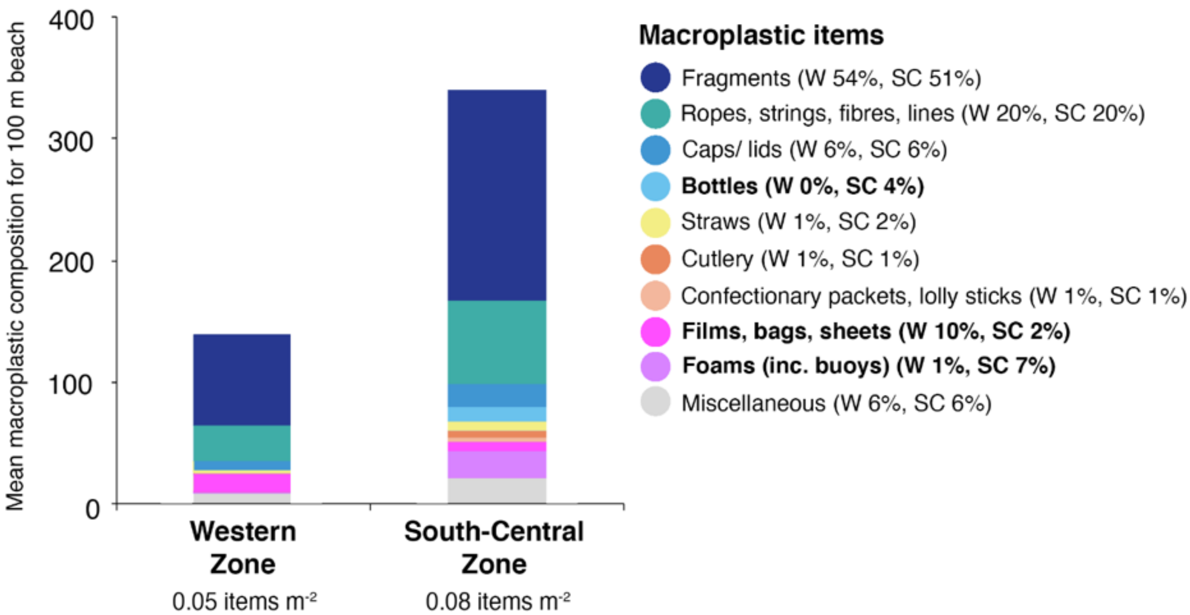


Figure 4.3: Mean macroplastic composition in each biogeographic zone. Data shown are counts per 100 m beach to present an average site in the Western (W) and South-Central (SC) Zones. Relative percentages of items are listed in the key with items driving significant differences in composition denoted with bold text.

Algae:macroplastic association

Several observations of interactions between macroalgae and large macroplastic debris were recorded in the South-Central Zone where macroalgae densities are lower but plastic presence is higher. Green algae (*Ulva* sp.) were recorded growing on a polyethylene carrier bag fragment at Punta Cormorán (Site 6) (Fig. 4.4a) and on the surface of a polypropylene bottle ring from Floreana harbour (Site 5) (Fig. 4.4b) showing attachment can occur on both soft and hard plastics. Two large Fish Aggregating Devices (FADs) were reported at Punta Cormorán (Site 6), one beached and one floating offshore (Fig. 4.4c). Both FADs were made of mixed plastics (net, ropes and sheeting) attached to a wooden frame and hosted rafting algal and invertebrate communities, primarily gooseneck barnacles (*Lepas* sp.), similar to those sampled in **Chapter 2**. A polypropylene fibre was also observed trapped in beached algae at the strandline at Lobería (Site 7) (Fig. 4.4d).

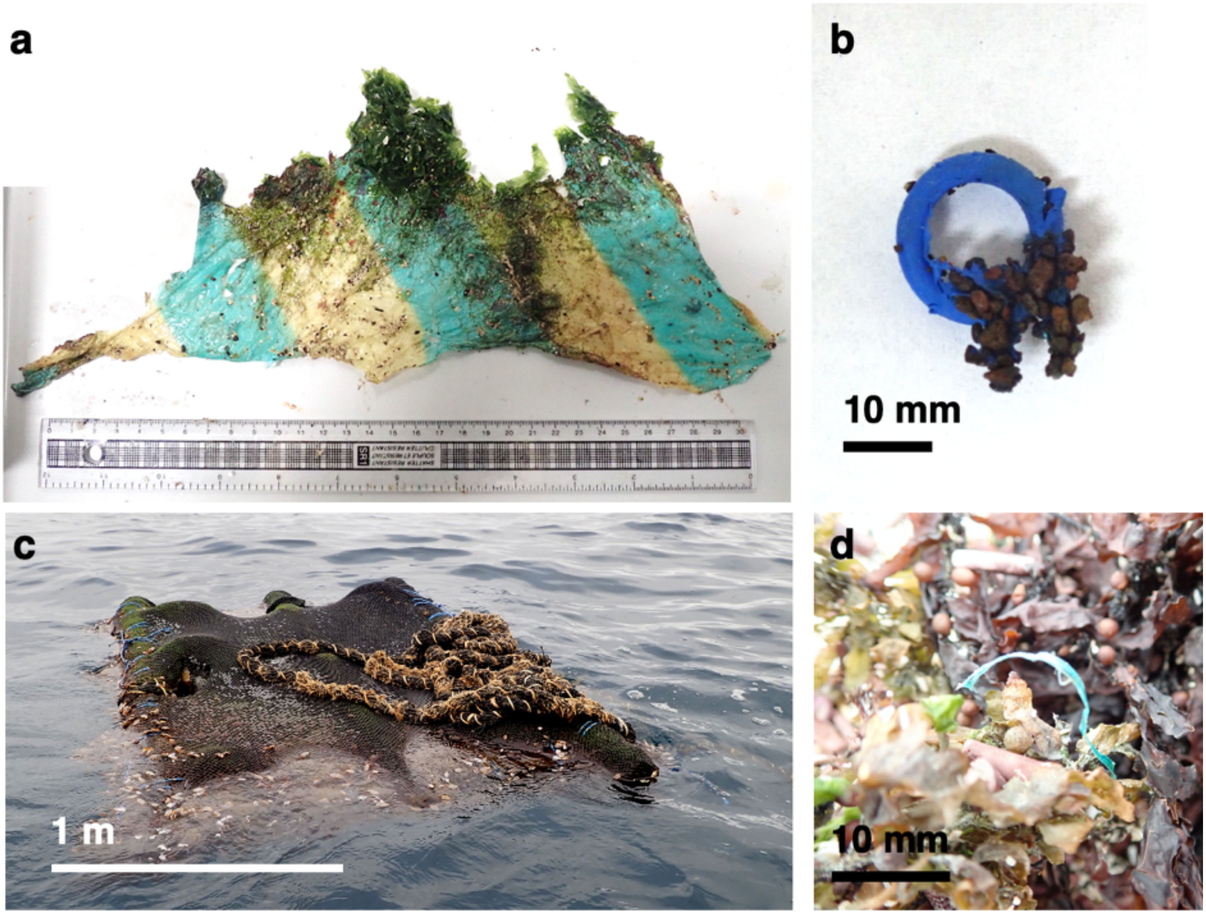


Figure 4.4: Observations of algae:macroplastic associations. (a) A plastic carrier bag fragment with green algae (*Ulva* sp.) (Site 6), (b) macroalgae growing on a bottle ring (Site 5), (c) a floating Fish Aggregating Device with visible algal and invertebrate colonies (Site 6), (d) a green fibre trapped among beached macroalgae at the strandline (Site 7). Photographs taken using an Olympus Tough TG-6 digital camera.

Polymer composition

Analysis by FTIR showed that macroplastic was primarily comprised of the low-density polymers: polyethylene and polypropylene (n = 140, 43% and 39% respectively, Fig. 4.5a). These two polymers also dominated the large microplastics sieved from beach sediment (n = 649, 23% polethylene and 54% polypropylene, Fig. 4.5b). In terms of shape composition, large microplastics were mostly fragments (86%) with the remainder pellets (9%) and fibres (5%). All pellets were recorded in the South-Central Zone (n = 57), primarily at Punta Cormorán (Site 6). The polymer composition of smaller particles in sand sediment (n = 22) was more difficult to determine. Four were polypropylene and polyethylene fragments (19%), four were a mixture of other plastic polymer fibres (19%) and one was natural (4%). Thirteen particles were unidentified with clear spectra not achieved (59%, all fibres). Overall, fragments were still more common than fibres in beach sand (64% and 36% respectively). Particles extracted across seawater surface samples (n = 69, Fig. 4.5d) were composed of polypropylene (26%, n = 19), one particle was suspected natural and 26 (39%) were unidentified due to lack of polymer match (again, all fibres). Fragments were marginally more common than fibres at the seawater surface (51% and 45% respectively) with films making up the remaining 4%. In benthic sediment (n = 39, Fig. 4.5e), blue polypropylene fragments were commonly found around Punta Cormorán (Site 6) (n = 19), five particles were other plastic polymers, nine particles were discounted as natural (calcium carbonate or chitin) and two blue fragments and nine blue fibres were unidentified. In terms of shape, 74% were fragments and 26% were fibres.

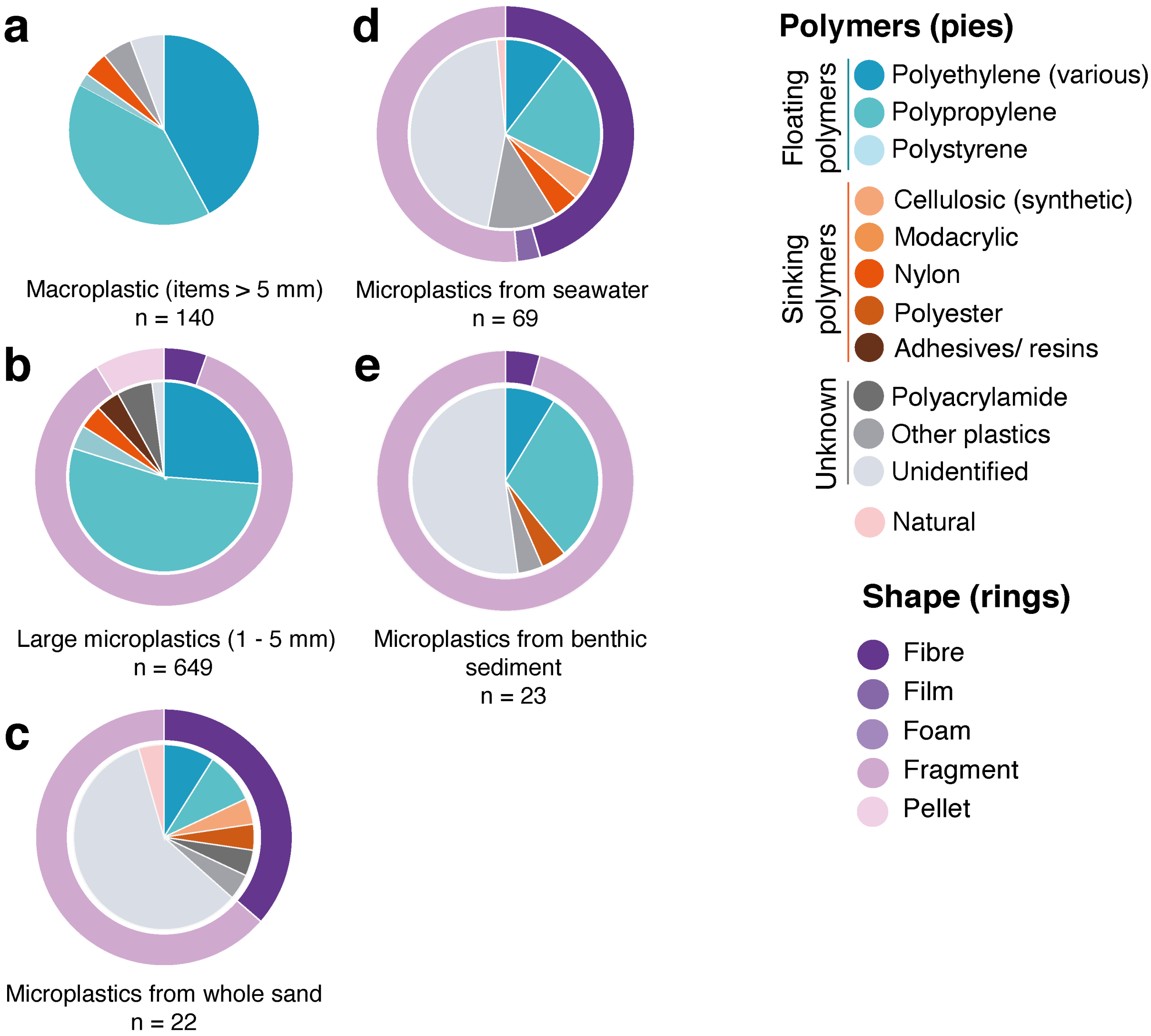


Figure 4.5: Polymer composition as verified by FTIR spectroscopy (inner pie charts) and shape composition (outer ring) of particles. Including (a) macroplastic, (b) sieved large microplastic, (c) beach sand, (d) seawater surface and (e) benthic sediment.

Macroalgae and microplastics

Of the 21 *Ulva* frond samples (Fig. 4.7a) comprehensively examined from eight sites across both biogeographic zones, 36.8% had synthetic particles associated with them (11.7% petrochemical polymer microplastics with the remaining 25.1% modified cellulosic polymers). Mean synthetic particle concentration was 0.38 ± 0.81 particles per g ww^{-1} or 0.11 particles cm^{-2} frond area (Fig. 4.6a). No significant difference was measured for algae:microplastic association between biogeographic zones (Wilcoxon's test, W = 52, $p = 0.5$). A total of 20 synthetic particles were recovered across all samples (18 fibres, 1 film, 1 fragment) (Fig. 4.6b). Fibres (mean length 1.62 mm) were comprised of blue cellulosics (Fig. 4.7b, n = 9), green polypropylene (Fig. 4.7c, n = 3), colourless cellulosics (n = 3), black polyester (n = 1) or unidentified black or colourless polymers (n = 3). The film fragment was very weathered polypropylene, mostly transparent (1.64 mm in diameter) (Fig. 4.7d) and the fragment was unidentified (n = 1). With the exception of one black polyester fibre collected from Albemarle (Site 1), all particles made of petrochemical polymers were sampled from Floreana Harbour (Site 5), the only populated site. There was no correlation with algae:microplastic contamination and any other habitat compartment including seawater, benthic sediment and beach when tested with Pearson's correlation although sample sizes were very small (Fig. 4.8). This analysis did however show that the abundance of microplastics sieved from the beach surface and beach sand samples had a very strong positive correlation (Pearson's Coefficient, r(df 7) = 0.97, $p < 0.001$), as did abundance of macroplastic and benthic microplastic, probably due to high levels of both at Punta Cormorán (Site 6) (Pearson's Coefficient, r(df 7) = 0.83, $p = 0.02$).

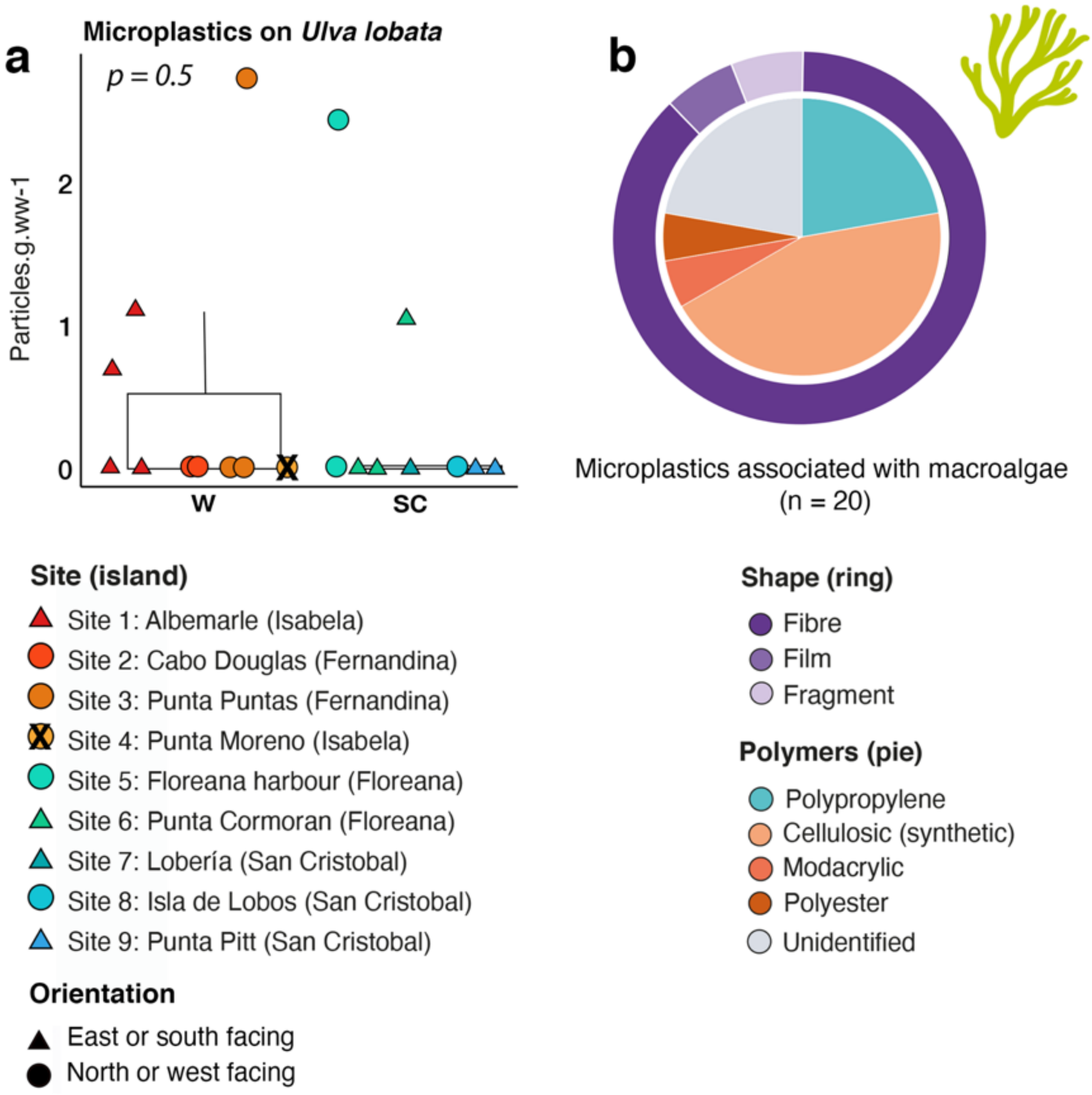


Figure 4.6: Algae:microplastic association. (a) Mean synthetic particles per gram wet weight of *Ulva lobata* across sampling sites. Bars denote mean. Sampling was not possible due to lack of *Ulva* growing at Site 4, denoted by X. (b) Polymer composition as verified by FTIR spectroscopy (inner pie charts) and shape composition (outer ring) of particles extracted from *Ulva lobata*.

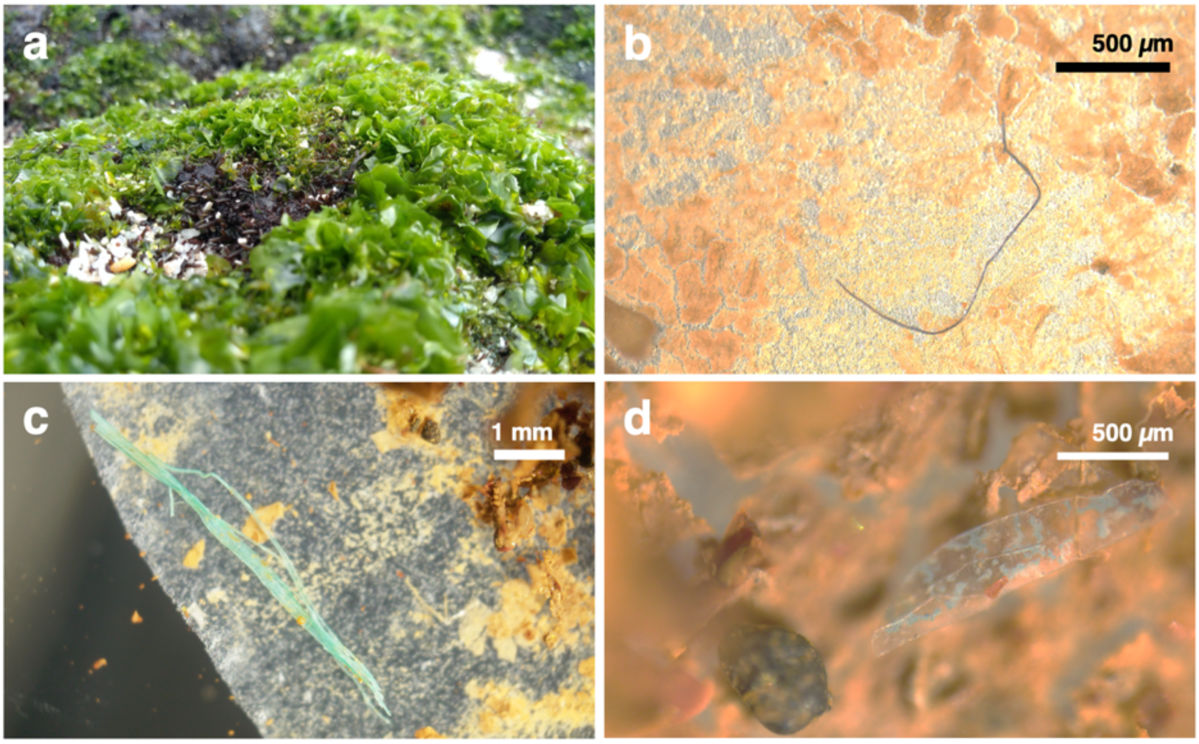


Figure 4.7: Algal study species and extracted microplastics. (a) *Ulva lobata* on littoral rocks (image taken using an Olympus Tough TG-6 digital camera), (b) a blue cellulosic fibre, typical of particles extracted from macroalgae samples across both zones, (c) green polypropylene fibres from macroalgae in Floreana harbour (Site 5) and (d) colourless/ blue polypropylene film fragment from macroalgae in Floreana harbour (Site 5) (b – d imaged using Olympus MVX10 microscope).

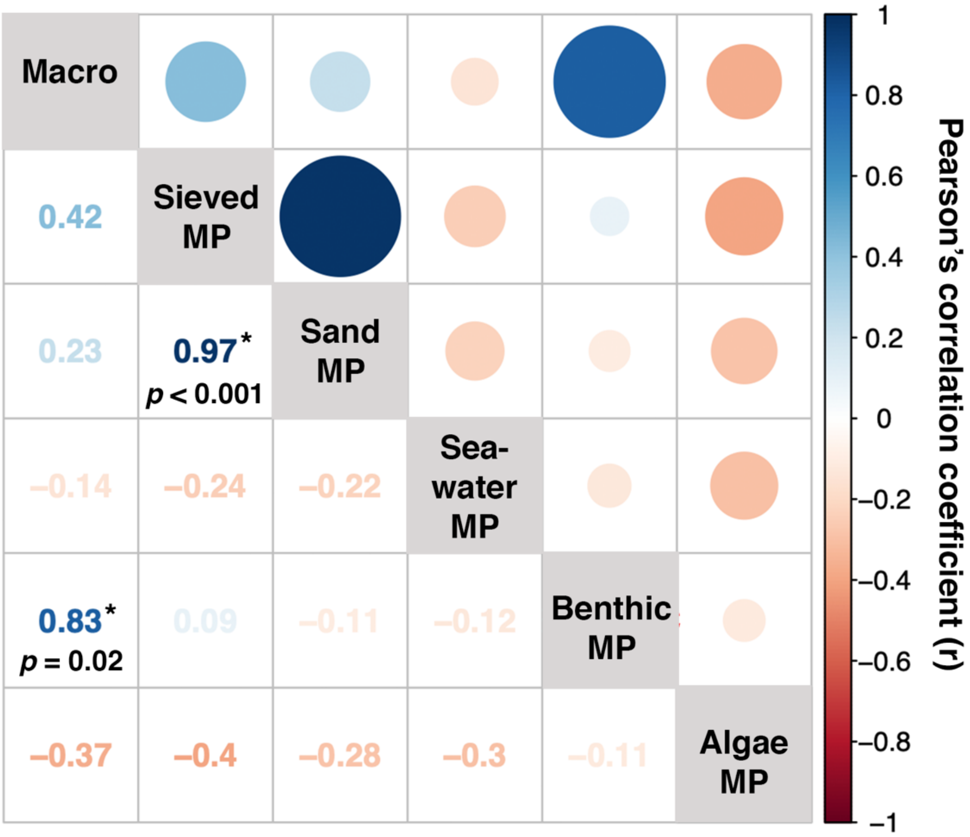


Figure 4.8: Correlation matrix for plastic abundance associated with environmental compartments and algae. Macro = beach macroplastic > 5 mm (items m^{-2}), Sieved MP = sieved microplastics 1 – 5 mm (particles m^{-2} in the top 50 mm), Sand MP = microplastics < 5 mm from whole sand beach samples (particles 50 g^{-1}), Seawater MP = seawater surface microplastics < 5 mm (particles m^{-3}), Benthic MP = microplastics < 5 mm from whole sediment samples (particles 50 g^{-1}), Algae MP = microplastics < 5 mm per g wet weight from *Ulva lobata* samples. Pearson's correlation coefficient (r) shown for each relationship in text in the same colour as the circle key, 1.0 = perfect positive correlation, - 1.0 = perfect negative correlation. Significant correlations denoted by * with *p* value reported.

4.4. Discussion

The unique oceanographic conditions of the Galápagos Marine Reserve that cause distinct biogeographic zones could also affect plastic movement and fate in the environment. The different ecology of each zone and the difference in abundance and composition of plastic contamination suggests that exposure of species is likely to vary, resulting in a different profile of ecological risk from macro- and microplastics throughout the marine reserve. These data support the hypothesis that plastic contamination is lower in the Western Zone than the South-Central Zone (six to ten-fold lower for microplastics) although contamination was present at all sites sampled. Algae:plastic association was observed at the macro and micro scale although no significant differences in interaction frequency were measured between zones and no correlation of algal contamination and contamination of different habitats was observed. This suggests that macroalgae is unlikely to be a useful bioindicator for monitoring microplastic contamination via the methodologies and sampling procedures described here. The strong correlation between sieved large microplastics (1 – 5 mm) and microplastics in whole beach sand samples could indicate potential degradation *in situ* but is not conclusive. Drivers for the correlation observed between beach macroplastic and benthic sediment contamination are less obvious. The highest contamination in both of these habitat compartments was observed at Punta Cormorán (Site 6) where patches of offshore rocks may be acting as barriers, accumulating microplastics that are washed off the beach by the tide in benthic sediments although again, this is not certain. The high variability between sites suggests that generalisations of plastic accumulation and food web incorporation in vegetated coastal areas should be used cautiously at this point as this field of research continues to develop, concurring with the findings of Cozzolino *et al.* (2020). Nevertheless, this study provides a useful first insight into the dynamics of plastic contamination across the Galápagos Marine Reserve at an Archipelago scale.

Spatial variation in plastic abundance between biogeographic zones

These data show that with the exception of large microplastics (1 – 5 mm) in beach sand, plastic contamination of Galápagos is relatively low compared to other oceanic islands (Lavers & Bond, 2017; Monteiro *et al.*, 2018; Pham *et al.*, 2020; Thiel *et al*., 2018). The significantly lower microplastic contamination in the Western Zone likely reflects (i) the lack of resident human population and low boat traffic, (ii) the effect of upwelling and offshore currents that transport surface waters away from the Archipelago (Van Sebille *et al.*, 2019) and (iii) the lack of large coastal accumulations of beached plastic debris. Plastic is washing up on western shores, however, as observed in Cabo Douglas (Site 2), on the western-most point of Fernandina island, which had the second largest accumulation of macroplastic (74% of which was hard fragments). Although this site is the closest to the strongest upwelling area, upwelling varies seasonally and therefore conditions may allow for occasional deposition of floating plastics, including during storm events as reported in the Azores (Pham *et al.*, 2020).

The distribution of beach macroplastic and microplastic > 1 mm was much more variable across sites than smaller particles, concurring with the hypothesis that different hydrographic and geophysical processes affect different size classes of plastics (Zhang, 2017). Larger items are easier to clean up and increasing community and Non-Governmental Organisation (NGO) efforts in the South-Central Zone at important visitor sites (particularly at Sites 5, 7 and 8) are likely to reduce macroplastic accumulations. The positive effect of cleaning interventions is also demonstrated by the regular citizen science 'Playas Sin Plásticos' surveys at Punta Pitt (Site 9). The cleaning effect of sample extraction has decreased the standing stock of larger plastics (> 5 mm) by 80% between May and September 2019, reducing concentrations from 0.46 items m^{-2} to 0.09 items m^{-2} (reported from the Playas Sin Plásticos project, **Chapter 5**). Without this cleaning effort, the difference might be more pronounced with the South-Central Zone more likely to accumulate greater volumes of plastic, increasing *in situ* fragmentation risk and microplastic burden. This is demonstrated by the highest density of macroplastic reported at the east-facing Punta Cormorán (Site 6) (0.25 items m^{-2}, 8.31 items m^{-1}) which is not regularly cleaned. This was less than a third of previously reported concentrations in Puerto Tablas however, on the east coast of San Cristóbal in May 2018 (0.66 items m^{-2}, 25.24 items m^{-1}; **Chapter 2**), highlighting this area as the most significant accumulation zone for macroplastics in the Galápagos Marine Reserve sampled so far.

Wastewater outfalls might be responsible for the high seawater surface concentrations of microplastics recorded at Isla Lobos (Site 8), an islet situated approximately 7 km north-east from the port of Puerto Baquerizo Moreno on San Cristóbal (population approx. 7,500), separated from the main island by a 125 m channel that may potentially be trapping this wastewater. The beach at this site was the cleanest however and so does not appear to be a depositional environment. This highlights the need for finer scale oceanographic models to understand how plastic moves within the environment, a tool that would also help to delineate sources. These models would also have useful application for establishing the potential movement of rafting invasive species, a conservation priority for Galápagos (Marras *et al.*, 2015; Izurieta *et al.*, 2018).

Characterising composition of plastic contamination in different biogeographic zones

Like many other oceanic islands (Lavers & Bond, 2017; Monteiro *et al.*, 2018), the majority of plastic items and particles on beaches were hard fragments from secondary sources, i.e. from the fragmentation of larger items (66% macroplastic and 86% microplastic < 5 mm). Fragments were generally polyethylene and polypropylene (82% macroplastic, 77% microplastic); floating, low density polymers that can be transported long distances from source (Gennip *et al.*, 2019; Lavers *et al.*, 2019). It is not known how much plastic arrives already fragmented and how much fragments *in situ*, but weathering is predicted to be elevated due to the equatorial location of the Islands causing higher levels of UV radiation (Andrady & Neal, 2009; Veerasingam, Saha, *et al.*, 2016) (explored further in **Chapter 6**).

Due to the degraded nature of the majority of plastic, it can be difficult to differentiate between continental waste management leaks and fisheries related sources. Some items are clearly linked with maritime industries (e.g. ropes, fishing gear, boat maintenance products and FADs) but with more 'domestic' items (e.g. drinks bottles, confectionary wrappers, combs, toothbrushes, cosmetic packaging) it is not easy to establish where these items entered the environment. Poor waste management and littering from fisheries, despite international agreements such as the International Convention for the Prevention of Pollution from Ships (MARPOL), have been linked with marine plastic contamination all around the world (Ryan *et al.*, 2019; Thiel *et al.*, 2018b; Unger *et al.*, 2017). Using Global Fishing Watch data (https://globalfishingwatch.org), it is possible to model fisheries presence around Ecuador's Economic Exclusive Zone (EEZ) which may support the future delineation of this input versus continental sources. When the sampling was undertaken for this study (August 2019), elevated fishery presence to the south-east of the Galápagos Marine Reserve due to international squid and tuna fleets (mostly Asiatic), suggests that littering was likely to be higher at this time of year, most likely affecting the South-Central Zone (Fig. 4.9a-b). In January – April, international fishing effort is highest to the West suggesting that less litter may enter the South-Central Zone from this source and perhaps is more likely to be washed up in the West due to the weakened upwelling, highlighting the need for further study to incorporate temporal differences.

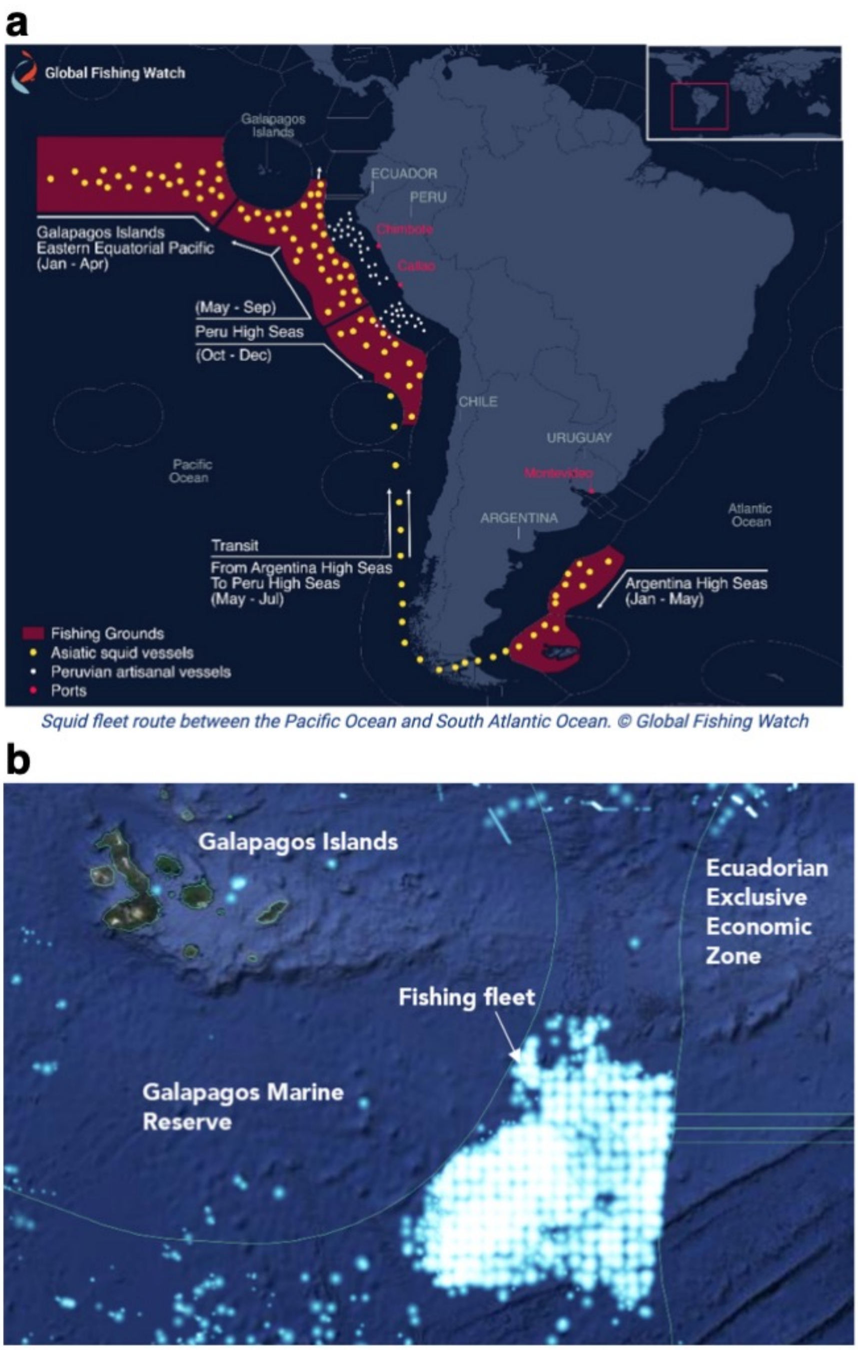


Figure 4.9: Annual fishery presence around the Galápagos Marine Reserve. a) Asiatic squid fleet annual fishery route showing presence to the South-East of the Galápagos Marine Reserve in May – September and to the west in January – April © Global Fishing Watch and b) Global Fishing Watch data for the month of August 2020 showing high density of Asiatic fishing fleet to the south-east of the Galápagos Marine Reserve, likely similar in 2019 at the time of sampling © Global Fishing Watch, modified by Galápagos Conservation Trust.

The composition of macroplastic items was statistically different between the biogeographic zones, driven by three specific item types. In the Western Zone, films/ sheeting represented a relatively higher percentage, generally associated with packaging or construction (e.g. tarp). This is not easy to explain due to the lack of human activity but perhaps could be due to the degradation of a few items over time. Drinks bottles were more frequently found in the South-Central Zone, likely due to the greater connectivity with tourism, continental waste management systems and fisheries operating to the South-East. Empty drinks bottles (generally made of PET) are very low mass and easily blown by the wind, particularly when lids remain attached (Ryan *et al.*, 2019). Those littered to the West may be more likely to be carried by winds and surface currents further West, towards the sub-tropical gyres as opposed to being beached along the shorelines of Galápagos.

Algae:plastic association

Synthetic particles (including petrochemical microplastics and modified cellulosics) were found associated with *Ulva lobata* throughout the Archipelago, at double the concentration recorded in Chinese mariculture farms growing *Ulva prolifera* (mean 0.38 particles $g.ww^{-1}$ in our study vs. 0.19 particles $g.ww^{-1}$) (Feng *et al.*, 2020). This could potentially be due to the morphology of the algae; *U. lobata* has large flat laminae compared to the long narrow laminae of *U. prolifera* that may affect particle settlement area. Particle counts per frond area also show ten-fold higher concentrations in Galápagos *U. lobata* (0.11 particles cm^{-2}) than in canopy-forming *Caulerpa* macroalgae in a Portuguese lagoon (0.012 particles $cm^{-2)}$ (Cozzolino *et al.*, 2020). Macroalgae was sampled from intertidal areas in our study, mostly from partially exposed rocks where wave action was high, representing a dynamic environment where microplastics might be temporarily trapped and then washed away from macroalgae fronds. Concurring with other macroalgae studies to date (Feng *et al.*, 2020; Seng *et al.*, 2018), fibres were most commonly found (18 out of 20 particles). Confirmed petrochemical microplastics included green polypropylene fibres that are commonly linked with maritime sources such as ropes. A greater number of larger macroalgae samples would be recommended for future sampling efforts, particularly focusing on populated areas or near wastewater outlets. In addition, laboratory exposures are required to establish the mechanisms of microplastic association to different species of macroalgae.

These data suggest that macroalgae represent a potential entry pathway for microplastics into the Galápagos food web via herbivorous grazers that indiscriminately consume particles associated with their algal diet. This pathway has been demonstrated by Gutow *et al.*, via the exposure of the common periwinkle (*Littorina littorea*) to brown macroalgae (*Fucus vesiculosus*) with fluorescent polystyrene fragments retained on its surface. This study showed uptake but the majority of fragments were egested in faeces with no significant retention nor harm recorded (Gutow *et al.*, 2016). Establishing the health effects of microplastic at a population level is complex, with effects on feeding, growth, reproduction and survival highly variable across taxa (Foley *et al.*, 2018; as described in **Chapter 3**). Additional ecotoxicological risk is presented by associated heavy metals and other POPs due to the strong affinity of macroalgae for high accumulations, reflective of environmental conditions (Mamboya, 2007). This is facilitated primarily by negatively charged polysaccharides in the cell wall that bind metals; a mechanism that has highlighted their potential as an environmental cleaning agent (Chan *et al.*, 2004; Salgado *et al.*, 2005).

Implications for marine iguanas

Due to their demonstrated sensitivity to POPs such as hydrocarbons due to their sensitive gut microbiomes (Wikelski *et al*., 2002), the marine iguana is a major species of interest for establishing potential impacts of microplastic contamination and associated POPs and pathogens. Further, this species is majorly impacted by El Niño events, where population crashes caused by changes in algal communities occur particularly in the western populations (Laurie, 1990); stress that may be exacerbated by the presence of plastics (both as an ingestion and entanglement risk).

Most iguanas feed on macroalgae from intertidal rocks although some larger adults dive to exploit sub-tidal algal beds. In their *Caulerpa* study, Cozzolino *et al.*, showed that microplastic association was higher in subtidal habitats than intertidal habitats (Cozzolino *et al.*, 2020) suggesting that additional sampling in the subtidal region in Galápagos would be of interest, perhaps to the common diving depth of marine iguanas (2 – 5 m). Feeding studies have shown that marine iguanas ingest everything associated with macroalgae scraped off rocks, evidenced by small stones up to 6 mm collected from stomach lavages. This suggests that if microplastics are present, they are likely to be indiscriminately ingested. The estimated weekly intake of fresh algae for an iguana (averaged over juvenile and adults) is 142 g (wet weight) with gut passage time estimated at 5 – 10 days using glass beads and plastic tags (Wikelski *et al.*, 1993). These particles used to estimate gut passage times are in the same size range as microplastics (1 – 5 mm) suggesting that egestion is probable after ingestion should an animal be exposed to rounded particles. This has also been assumed in sea turtles (Duncan *et al.*, 2019). Fibres (most commonly found in this study) may behave differently in organisms however, potentially more likely to be retained which also suggests size and shape of microplastics are important considerations for linking to potential health effects (Nelms *et al.*, 2019; Welden & Cowie, 2016a).

Taking the mean synthetic particle abundance per wet weight gram of *U. lobata*, an average iguana might be exposed to 54 microplastic particles per week from their algal diet (using the 142 g average intake estimated by Wikelski *et al.* mentioned earlier), likely to be higher if their grazing range is close to human populations. It is important to note that *U. lobata* is not the sole diet of marine iguanas and species-specific differences in macroalgae such as variation in physical morphology, electrostatic surface charge, mucus layer and biological properties of the vegetation surface might affect attachment likelihood and thus microplastic accumulation (Goss *et al.*, 2018; Gutow *et al.*, 2016). Nevertheless, these results represent an important finding that marine iguanas are likely exposed to microplastics through their diet and with global plastic contamination estimated to treble in the next twenty years (Lau *et al.*, 2020), this could be an increasing threat to this already vulnerable species.

4.5. Conclusion

Here I demonstrate that the distribution and composition of plastic contamination varies between biogeographic zones of the Galápagos Marine Reserve, pointing to a variety of sources and therefore intervention points. The Western Zone is less contaminated than the South-Central Zone but plastic contamination is nevertheless present in this region. Potential risks to the Galápagos marine food web are likely to vary between zones but further sampling is required to capture a finer degree of spatial and temporal variation to support the identification of drivers of plastic accumulation. There is an emerging trade-off between targeting efforts for plastic mitigation in high biodiversity or endemism hotspots where overall contamination is lower but encounter rate might be higher, or in areas where plastic accumulates in the highest volumes. From a conservation perspective, high species endemism makes the Western Zone an important region for protection. Several of the top scoring vertebrate species in the prioritisation analysis (**Chapter 2**) are either found exclusively in this area e.g. the flightless cormorant (*Phalacrocorax harrisi*) or the majority of their population is e.g. the Galápagos penguin (*Spheniscus mendiculus)* and the Galápagos fur seal (*Arctocephalus galapagoensis*). Further investigation is required to better understand the role of marine vegetation in plastic pathways and fates at a global scale. To address these knowledge gaps, a multi-disciplinary approach incorporating ecology, oceanography and ecotoxicology is recommended.

4.6. Acknowledgements

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Chapter 5

Microplastic contamination on two Galápagos Island beaches, Ecuador: An assessment using citizen science derived data

This chapter is a reformatted version of a manuscript in preparation: **Jen S. Jones**, Anne Guézou, Sara Medor, Caitlin Nickson, Georgina Savage, Daniela Alarcón-Ruales, Tamara S. Galloway, Juan Pablo Muñoz-Pérez, Sarah E. Nelms, Adam Porter, Martin Thiel and Ceri Lewis. Microplastic contamination on two Galápagos Island beaches, Ecuador: An assessment using citizen science derived data.

Contributions: Conceptualisation of the study, writing of the manuscript, data analysis, visualisation and funding acquisition was led by J.J..
Methods were co-developed by J.J., A.G., S.M., A.P. and C.L..
Fieldwork was undertaken by S.M., A.G., D.A.R., J.M.P. and J.J..
Project supervision support was provided by T.S.G. and C.L..
Laboratory analysis was done by J.J. and C.N..
The manuscript was edited by S.N. and A.P. and the final manuscript shared with all authors for review.

Microplastic contamination on two Galápagos Island beaches, Ecuador: An assessment using citizen science derived data

Abstract

Monitoring beach plastic contamination across space and time is a prerequisite for understanding ecological effects, but data collection often represents logistical and financial challenges, especially for microplastics. Citizen science represents a potential data collection option, but challenges persist around sampling and accuracy. We designed a simple methodology using a standard quadrat unit (50 cm x 50 cm x 5 cm) sieved from the surface layer of sand by citizen scientists to support a detailed analysis of spatiotemporal trends in beach microplastic contamination on two islands in the Galápagos Archipelago (San Cristóbal and Santa Cruz). Student volunteers (aged > 15 years) undertook sampling, supervised by trained coordinators between April 2019 and January 2020. We tested identification accuracy of microplastic (synthetic vs organic) by comparing their results with the output of polymer analysis using Fourier-transform infrared spectrometry of 2,192 particles (~30% total sample). This confirmed that visual identification of beach microplastics and rubber fragments > 1 mm was 93% accurate. Over the two beaches, concentrations ranged from zero to > 2,500 particles m^{-2}. A strong accumulation gradient was measured; perpendicular to the waterline in Tortuga Bay (higher in sea turtle nesting habitat than at the strandline) and parallel to the waterline at Punta Pitt (five-fold higher concentrations in the south vs the north) suggesting different hydrodynamic and geophysical drivers are affecting accumulation. No significant temporal differences in microplastic concentrations were measured. The majority of microplastic was from secondary sources (90%), comprised of polyethylene and polypropylene fragments. In Tortuga Bay however, 24% of particles were variably weathered polyethylene pellets, evidencing contamination by primary microplastics. Our results demonstrate the value of citizen science in studying beach microplastics, employing a standard sampling unit but encouraging complementary student-led investigations where

appropriate. We demonstrate the value in adding weathering scoring and biofouling identification to supplement future studies.

5.1. Introduction

Sandy beaches make up 31% of the Earth's ice-free coastline, support diverse biological communities and have a major socioeconomic importance for tourism and recreation (Defeo *et al.*, 2009; Luijendijk *et al.*, 2018). At the interface between terrestrial and marine ecosystems, beaches provide many essential ecosystem services including sediment storage, water filtration, nutrient cycling and provision of important habitats for many endangered species, including sea turtles and pinnipeds (Defeo *et al.*, 2009). The accumulation of litter on beaches is reported globally, with plastic debris often representing 60 - 80% (Davis & Murphy, 2015; Gaibor *et al.*, 2020; Thiel *et al.*, 2013; Watts *et al.*, 2017). Plastic debris may originate from a variety of sources in different ratios depending on the site. Plastics may wash up on the tide, often transported for long distances from source by oceanic currents, blow onto the beach from terrestrial inputs, or be littered *in situ* by beach visitors (De-la-Torre *et al.*, 2020; Serra-Gonçalves *et al.*, 2019; Yu *et al.*, 2016). Negative impacts to marine species from interactions with plastic contamination have been reported across a wealth of taxa from plankton to whales, with impacts ranging from mortality caused by entanglement, to disease, inflammatory responses and false satiation that affect scope for growth (Alexiadou *et al.*, 2019; Cole *et al.*, 2015; Wright *et al.*, 2013).

Microplastics (particles < 5 mm) are of particular concern for coastal food webs due to their small size and resulting wide bioavailability to a suite of species. Although entanglement in larger plastic debris may be more likely to be lethal to organisms than ingestion of microplastics (Wilcox *et al.*, 2016), sub-lethal impacts from chemical contamination may pose an ecotoxicological risk to exposed beach biota, enhanced by the aptitude of plastic to adsorb hydrophobic persistent organic pollutants (POPs) including polychlorinated biphenyls (PCBs)

(Ogata *et al.*, 2009), heavy metals (Brennecke *et al.*, 2016), antibiotics (Liu *et al.*, 2020) and pathogens (Bowley *et al.*, 2021).

The identification of plastic accumulation zones is fundamental to establishing risks to beach biota and focusing clean-up efforts. The movement of plastics throughout the beach environment is affected by anthropogenic drivers such as waste management leaks, littering and clean ups in addition to geophysical and hydrographic features, such as prevailing ocean currents, offshore bathymetry, tidal dynamics, beach grain size and beach slope (Derraik, 2002; Hardesty *et al.*, 2016). The dynamic connection with the nearshore marine environment means that microplastics may be in flux between floating on the sea surface, in suspension in the water column and being beached, or buried in benthic sediments meaning that the quantification of standing stock is a major challenge (Zhang, 2017). Biological processes also impact the environmental pathways of microplastic creating temporary sinks, for example by ingestion and subsequent egestion and sequestration in faeces (Katija *et al.*, 2017), bioturbation (Gebhardt & Forster, 2018) and integration into biological structures e.g. polychaete tubes (Nel & Froneman, 2018). Seasonal changes may also affect accumulation, in addition to episodic transitional processes such as storm surges or tectonic events (Walsh *et al.*, 2016).

Gathering the scale of spatiotemporal information needed to understand the distribution and composition of beach microplastic to account for this variability is a major challenge, particularly for remote areas with limited access. The Galápagos Islands (Ecuador), are a highly protected UNESCO World Heritage Site in the Eastern Tropical Pacific (01°40’N - 01°25’S, 89°15’W - 92°00’W), famous for their endemic biodiversity and dependent on their ‘pristine’ image to maintain the tourism industry that supports > 80% the local economy (Pizzitutti *et al.*, 2017). Currently, the Eastern Pacific region has very little plastic monitoring data, including for beaches (Serra-Gonçalves *et al.*, 2019), seawater surface microplastics (Van Sebille *et al.,* 2015) and negative wildlife interaction records (Nelms *et al.*, 2016). There is international research effort to reduce the impacts of plastic waste in the sensitive ecosystem of the Galápagos Marine Reserve but as yet, no temporal data exists to explore seasonal patterns or long-term trends in beach microplastics.

Citizen science is increasingly recognised as a powerful, change-making tool to generate and analyse long-term datasets; making the scientific process accessible to a growing, participatory audience (Bonney *et al.*, 2009; Rambonnet *et al.*, 2019). Citizen science has played a major role in plastics monitoring around the world in the last several decades, primarily focused on beaches due to accessibility and public appeal (Nelms *et al.*, 2017; Serra-Gonçalves *et al.*, 2019) although riverine studies have increased recently, connecting urban audiences with the issue (Emmerik *et al.*, 2020). In addition to filling spatiotemporal knowledge gaps of plastic movements in the environment, these data can be useful to highlight management priorities such as in a study of Marine Protected Areas in the United Kingdom where sewage related items next to large rivers and estuaries pointed to clear sources (Nelms *et al.*, 2020).

Citizen science studies for microplastics are less common due to the difficulty of sample extraction and verification of small microplastics, generally enforcing a lower limit of 1 mm due to the need for visibility (Davis & Murphy, 2015). Many microplastic citizen science projects focus solely on industrial pellets (nurdles) on the surface of beaches (e.g. Tunnell *et al.*, 2020), reducing the logistical issues of sample extraction and difficulties differentiating plastic from other materials including natural sediments (Schmuck *et al.*, 2017; Zettler *et al.*, 2017). A six-hour Nurdle Hunt by 16 citizen scientists at Tortuga Bay, Santa Cruz, Galápagos in February 2019 (one month before our survey began) yielded 9,000 pellets, a major impetus for our site selection (www.nurdlehunt.co.uk; last accessed 08 January 2021). Some studies undertake all sample processing in the laboratory, only requesting the collection of sand samples by citizen scientists, therefore ensuring better quality assurance and quality control during sample processing, affording the ability to investigate smaller particles < 1 mm and incorporating high density polymers that may not be possible to extract in the field (e.g. Lots *et al.*, 2017). The other common alternative for beach microplastic sampling is *in situ* beach sieving, excavating a quadrat to a standard depth of surface sediment and retaining the microplastic fraction for later analyses (e.g. Hidalgo-Ruz & Thiel, 2013; González-Hernández *et al.*, 2020).

The lack of harmonised data collection in plastics research is an ongoing issue (Hartmann *et al.*, 2019) but the use of a standard unit helps to unify projects and facilitate monitoring efforts over a longer term and a wider spatial scale. We adopt this idea to enable comparison and also a scalable option for future surveys. Additional hypotheses may be added by citizen scientists to support engagement and provide scientific training. Using the samples collected, we identified two further data categories that could be potentially added to future surveys: weathering scoring and biofouling identification. The degree of weathering experienced by a particle will affect how it moves around the environment, its surface area for potential adsorption of persistent organic pollutants and pathogens, its fragmentation potential generating more microplastics, and demonstrates the aging of particles to support the determination of sources (Veerasingam *et al.*, 2016). Biofouling is of major interest in the Galápagos Islands where non-native marine species entering the marine reserve on floating plastic debris pose potential invasion risks for sensitive ecosystems (Keith *et al.*, 2016).

The main aims of this study were: (i) to test student citizen science data collection methods as an effective approach for monitoring the distribution and composition of beach microplastic, verifying the accuracy of visual identification by spectroscopy, (ii) to measure spatiotemporal microplastic variation on two beaches over a year to capture potential seasonal differences, incorporating student research interests and (iii) to test visual analysis of surface particle characteristics, including weathering scoring and bio-fouling, as a future addition to citizen science protocols.

5.2. Methods

Study sites

This study focused on two beaches in the Galápagos Archipelago, Ecuador. Tortuga Bay on Santa Cruz (0.7657°'S, 90.3335°'W, Fig. 5.1a, Fig. 5.1c) is a Galápagos National Park visitor site with public access, approximately 45 minutes' walk from Puerto Ayora, the largest town in Galápagos (population approx. 16,000) (INEC, 2010). The sampled area, known as Playa Brava, is a wide, south-facing, gently sloping, dissipative beach exposed to southern swells, ~ 1.4 km in length and composed of very fine, loose, white sand (Supplementary Figure 5.1). The dune at the back of the beach represents a nesting area for the Galápagos green sea turtle (*Chelonia mydas* subpopulation) (Carrera *et al.*, 2019). The site is popular with surfers and local day trippers and is cleaned almost daily by Park Rangers meaning that macroplastic accumulations are rarely observed. In contrast, Punta Pitt beach on San Cristóbal (0.7116°'S, 89.2528°'W, Fig. 5.1a, Fig. 5.1d) has no public access and is not cleaned. It is a narrow, east-facing, intermediate-reflective beach, < 0.5 km in length and composed of fairly compact, fine grain, olivine sand (Supplementary Figure 5.2). This area is of high conservation management priority due to its importance for endemic and endangered species including the rarest marine iguana subspecies in the Archipelago (*Amblyrhynchus cristatus godzilla*) with an estimated population of just 400 individuals (Miralles *et al.*, 2017).

Citizen scientist recruitment and coordination

Two survey coordinators (qualified biologists) were trained in survey methods over a week in the field before citizen science surveys began. Sampling was managed by one coordinator for each site to ensure consistency and increase reliability of data. A small team of volunteers in the UK supported the analysis of microplastic photographs from Punta Pitt, following an online training session in data entry and categorisation. The Tortuga Bay surveys were undertaken by the Mola Mola eco-club and high school students enrolled in the Galápagos National Park Directorate's environmental education course (aged 15 - 18 years). The Punta Pitt surveys were undertaken by local university students aged > 18 years based at the Galápagos Science Center campus of the Universidad San Francisco de Quito on San Cristóbal island.

Playas sin Plastics protocol: sampling beach microplastic

The basic 'Playas sin Plásticos' ('Beaches without Plastics') methodology (Fig. 5.1b) employs a standard unit compatible with the United Nations' GESAMP and the European Commission's Marine Strategy Framework Directive recommendations (European Commission, 2013; GESAMP, 2015) represented by a quadrat of known size sampled to 50 mm depth, at a known location and time, at the strongest visible strandline on the beach. Kits were provided including laboratory grade stackable sieves (1 mm and 5 mm), quadrats (50 cm x 50 cm), a measuring tape (50 m), bucket, tweezers, protocols and datasheets (see Supplementary materials). Educational activities were developed, including resources such as a photographic identification guide for common microplastics to avoid mis-identification (as recommended by Hidalgo-Ruz & Thiel, 2013). The datasheet was completed as a group at the beginning of every survey incorporating meteorological information, beach characteristics and human/wildlife observations.

Students were divided into groups of 2 – 4 to sample quadrats as per their study design. The location of each quadrat was recorded using a Global Positioning System device (handheld Garmin Etrex 10). Large organic debris, such as vegetation, was removed from the surface of the quadrat and any visible microplastics were picked off by hand or using tweezers and retained in a plastic tube with lid or paper bag. Beach surface sediment was excavated by hand or using a metal shovel to 50 mm depth and sieved through stacked 5 mm and 1 mm sieves. To ease sieving and encourage the flotation of microplastics away from natural sediments, the sieve stack was gently submerged into a bucket of seawater that had been visually checked for floating debris before use. To further reduce the likelihood of contamination of the samples from the seawater, the water level was not allowed to exceed the height of the top sieve. This basic field-based density separation method replicates that which is often used in the lab to extract microplastics from sediments (Coppock *et al.*, 2017) (albeit at a lower extraction potential as the method is predicated by the density of the floatation media). Suspected plastics were removed from the water surface within the sieve, or from the sieve itself, and categorised by type and colour by the citizen scientists. In terms of contamination control, as small fibres were not included in the analysis, no atmospheric contamination control was collected in the field. In the laboratory, normal quality control procedures were employed such as thorough cleaning of all equipment and surfaces, wearing of a cotton lab coat and keeping samples covered when not in use.

Study design

In Tortuga Bay, two sampling campaigns took place to compare seasonal differences in March/ April 2019 and July/ August 2019 (total quadrats = 40). Due to the large size of the beach, quadrats were sampled every 100 m across a distance of 1 km to ensure a representative concentration across the entire beach. Citizen scientists decided to investigate the difference in microplastic concentration between the highest strandline and the turtle nesting line (close to the base of the back-beach dune, 2 – 5 m further up the beach) (Fig. 5.1c). All particles collected were retained in a paper bag for each quadrat to facilitate laboratory recounts, imaging and polymer analysis.

In Punta Pitt, citizen scientists aimed to test patterns in microplastic distribution across two fixed 50 m transects in the north and south of the beach over the course of a year. Nine surveys were undertaken between April 2019 and January 2020, after which any subsequent planned fieldwork was cancelled due to COVID-19 restrictions. Three quadrats (50 cm x 50 cm x 50 mm deep) were sampled at the strandline for each transect, a total of six quadrats per survey (total quadrats = 54) (Fig. 5.1d). Suspected microplastics were photographed on a sheet of white paper with a ruler for scale and a subsample (n = 500, approx. 10%) was kept for later analysis.

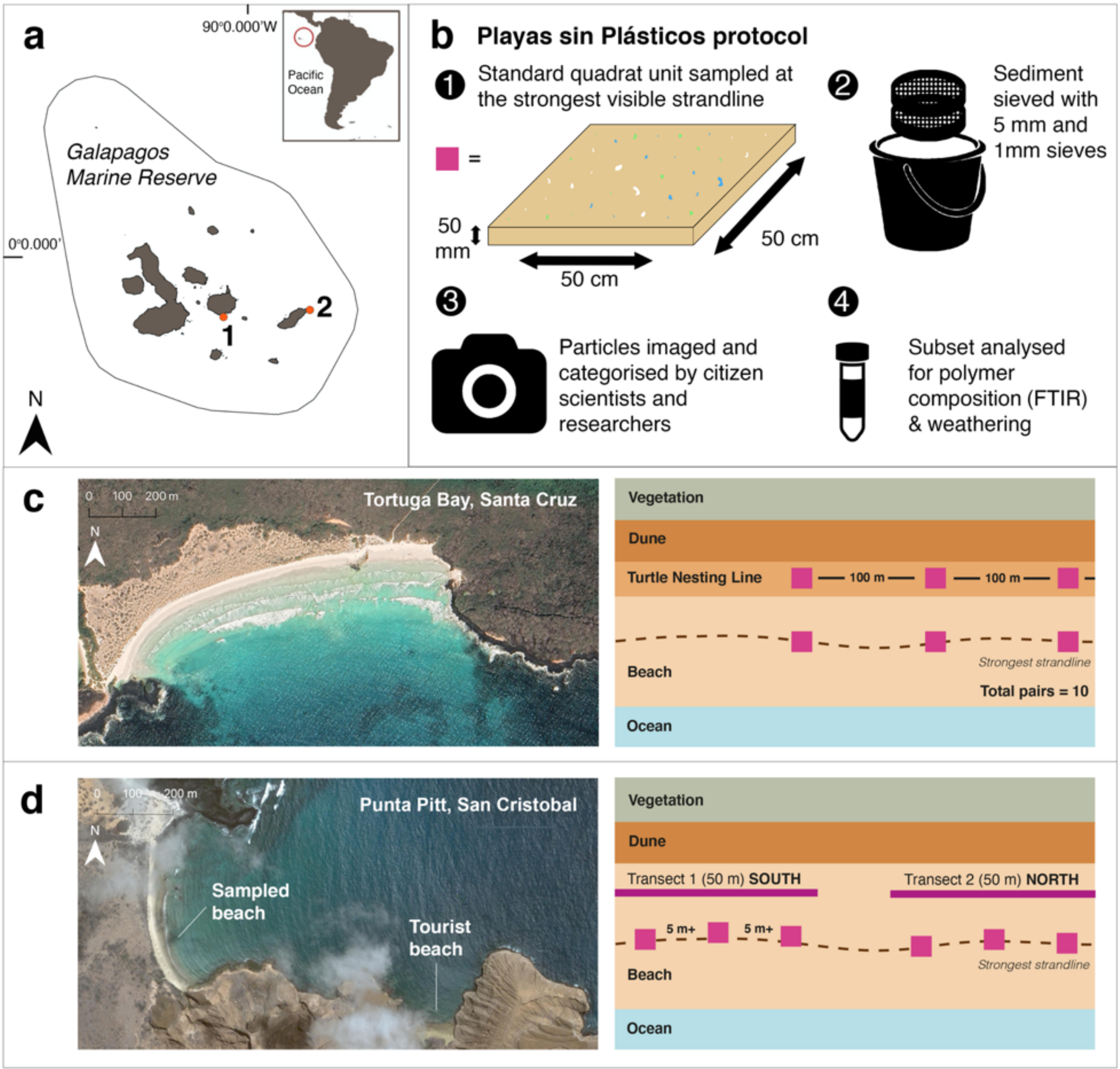

Figure 5.1: Playas sin Plásticos sampling protocol and study design. (a) Map showing the geographic location of the two citizen science surveys (Tortuga Bay (1) and Punta Pitt (2)), (b) graphic representation of the standard Playas Sin Plásticos beach microplastic sampling protocol, (c) satellite image of Tortuga Bay with sampling strategy of pairs of quadrats at the strandline and turtle nesting line every 100 m along the beach, (d) a satellite image of Punta Pitt with sampling strategy of three quadrats per fixed 50 m transect, one either side of the beach. Satellite images retrieved from Google Earth Version 6.2. (accessed 18 February 2020), Galápagos Islands, http://www.earth.google.com

Testing student accuracy

Microplastic sampling was closely supervised by the coordinators who checked student quadrats and counts in the field. Each project was visited twice during the project period by the first author (JJ). We tested the identification ability of students to differentiate between microplastics and other materials by comparing the citizen science results with the output of polymer analysis using Fourier-transformed infrared (FTIR) spectroscopy. All suspected plastic particles from Tortuga Bay over the entire survey (n = 1,717) and a subsample from Punta Pitt (n = 500) were analysed using a Cary 630 FTIR (Agilent Technologies, CA) in single reflectance Attenuated Total Reflectance (ATR) mode. Spectra were measured in the range of wavelength from 650 – 4,000 cm^{-1} with a resolution of 4 cm^{-1} and averaged over 4 scans. The threshold for minimum hit quality between the absorbance spectra to those in the standard Agilent polymer reference library was 70%. Due to the conservation status of our study sites, it was not feasible nor ethical to extract the entire excavated quadrats to assess accuracy of the extraction methodology via laboratory recounts of the same sediment. This has been tested previously however in a similar study with 1,000 school-age citizen scientists in Chile, where results were not significantly different for supervised students to that of the researchers (Student's t-test (t = -1.517, df = 31, p = 0.139) (Hidalgo-Ruz & Thiel, 2013). To assess reporting accuracy of their data, all samples (stored in paper bags) from Tortuga Bay were recounted and categorised in the laboratory in addition to all photographs of all Punta Pitt samples.

Surface characteristics and weathering of microplastics

To add further value to citizen science microplastic data, we used the collected particles to develop a simple weathering scoring system to assess if this additional visual analysis could be added to future citizen science studies. All particles analysed by FTIR (n = 2,192) received a weathering score between 1 and 5 based on a combination of visual characteristics and behaviour under the pressure exerted from the probe during analysis (see Supplementary

Table 5.1 for scoring criteria). Scanning Electron Microscopy (SEM) images of a small subset of pellets (n = 10) were collected to investigate surface weathering at the microscopic scale to compare with assigned visual weathering scores. Samples were placed on double-sided carbon tape atop an aluminium plate for analysis using a Q150T ES Sputter Coater (Quorum, UK) with Au/Pd 10nm (80:20) coating material at a current of 20mA and a GeminiSEM 500 (Carl Zeiss, Germany) producing images at x477, x5,000 and x10,000 magnification. The subsamples from Punta Pitt (n = 500) were also visually analysed (by the naked eye) for evidence of biofouling (presence/absence).

Data Analysis

All statistical analysis was undertaken using RStudio Version 1.3.1093 (R Core Team, 2020). Regression analysis was undertaken to investigate the relationship between student and scientist particle counts of extracted samples (checking reporting accuracy). Following citizen science data validation via polymer analysis, a conversion factor was applied to the remaining suspected plastic particles that were not verified by FTIR. This was calculated by the percentage accuracy of plastic identification by the students across both projects. Normality of the data was assessed visually using a Q-Q plot and via the Shapiro Test. Data were deemed non-normal and a nonparametric Kruskal-Wallis test was chosen to test variance of means between multiple groups. Wilcoxon Rank Sum Tests were used to compare means between sides of the beach, strandline vs turtle nesting line and between seasons. The null hypothesis was rejected if $p \leq 0.05$. Microplastic distribution was mapped using QGIS version 3.16.2 using satellite imagery retrieved from Google Earth.

5.3. Results

Testing student accuracy

Over 50 school and university students (aged 15 – 25 years) participated across the two projects over 10 months. Particle analysis by FTIR confirmed that citizen scientists accurately identified 93% of particles as plastics (90%) or synthetic rubbers (3%) across the two study sites (n = 2,192) (Fig. 5.2a). The remaining 7% comprised of natural particles (5%, n = 110), primarily calcium carbonate shell fragments or chitinous crustacean carapaces, or were unidentified due to lack of spectra match (2%, n = 46). A conversion factor of 93% was therefore applied to all photographic data from Punta Pitt of samples that were not verified by FTIR to account for potential misidentification (5,118 particles). Laboratory recounts of extracted particles from Tortuga Bay (n = 1,717) showed that student reported counts and categorisation were highly accurate with a significant positive correlation (Fig. 5.2b, $F(1,39)$ = 3,259, $p < 0.001$, $R^2 = 0.98$). The lower particle count was used for each quadrat sample to control for suspected fragmentation in transit (three samples) or suspected over-counting by students (three samples). For Punta Pitt, 18 particles were rejected after re-analysis of photographs (5,136 particles) due to identification as invertebrate carapaces.

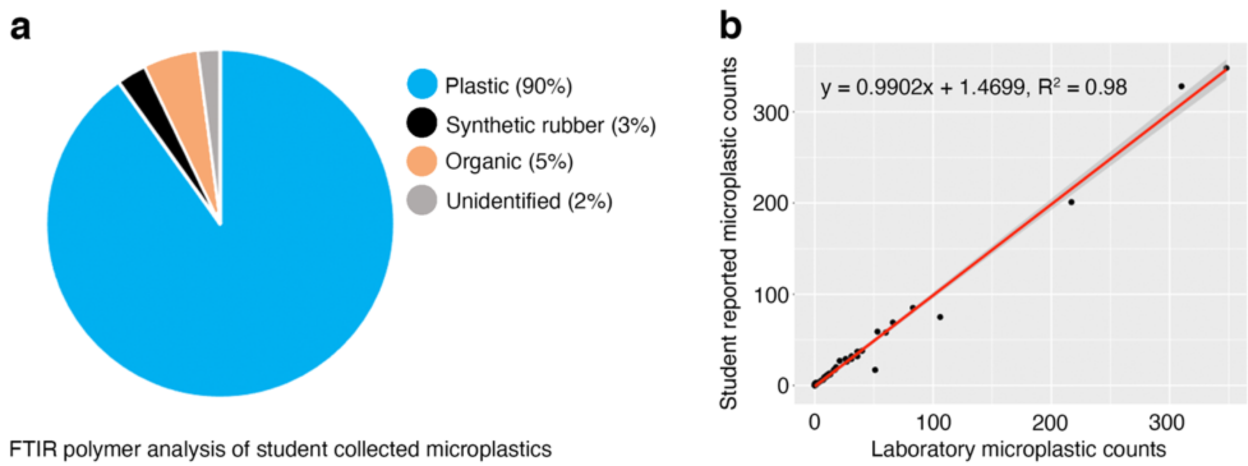

Figure 5.2: Verification of accuracy of student citizen scientists. (a) Polymer verification by FTIR analysis of composition of suspected microplastics (1 – 5 mm) from citizen science data across two beaches in Galápagos (n = 2,192); (b) regression analysis of student vs laboratory counts for samples extracted from 40 quadrats collected from Tortuga Bay, Santa Cruz in 2019.

Tortuga Bay: Distribution and composition of beach microplastic

At the strandline, the mean concentration of microplastic ± SD was 95 ± 56 particles m^{-2} on the eastern side of the beach and 58 ± 20 particles m^{-2} on the western side (Fig. 5.3a). Concentrations were significantly higher at the turtle nesting line (Wilcoxon Rank Sum Test, $W = 291$, $p = 0.03$), particularly in the east where mean particle abundance was > 400% higher (440 ± 167 particles m^{-2}). High variability meant no spatial differences between the east and west beach were statistically significant across the full study timescale (Wilcoxon Rank Sum Test, $W = 262.5$, $p = 0.12$). The mean concentration of microplastics were 48% higher in the dry season, although this was not statistically significant (Wilcoxon Rank Sum Test, $W = 154$, $p = 0.15$). Of the 1,692 particles analysed, the majority were hard fragments (70%, mean size 4.3 ± 3.2 mm) but pellets (mean size 4.2 ± 0.8 mm) were also common, representing 24% of the sample (Fig. 5.3b). The majority of pellets (92%) were colourless, white or yellowing polyethylene and were especially common at the turtle nesting line (95% of all pellets found there). Overall, the low density polymers polyethylene and polypropylene were most common (69% and 15% respectively) (Fig. 5.3c).

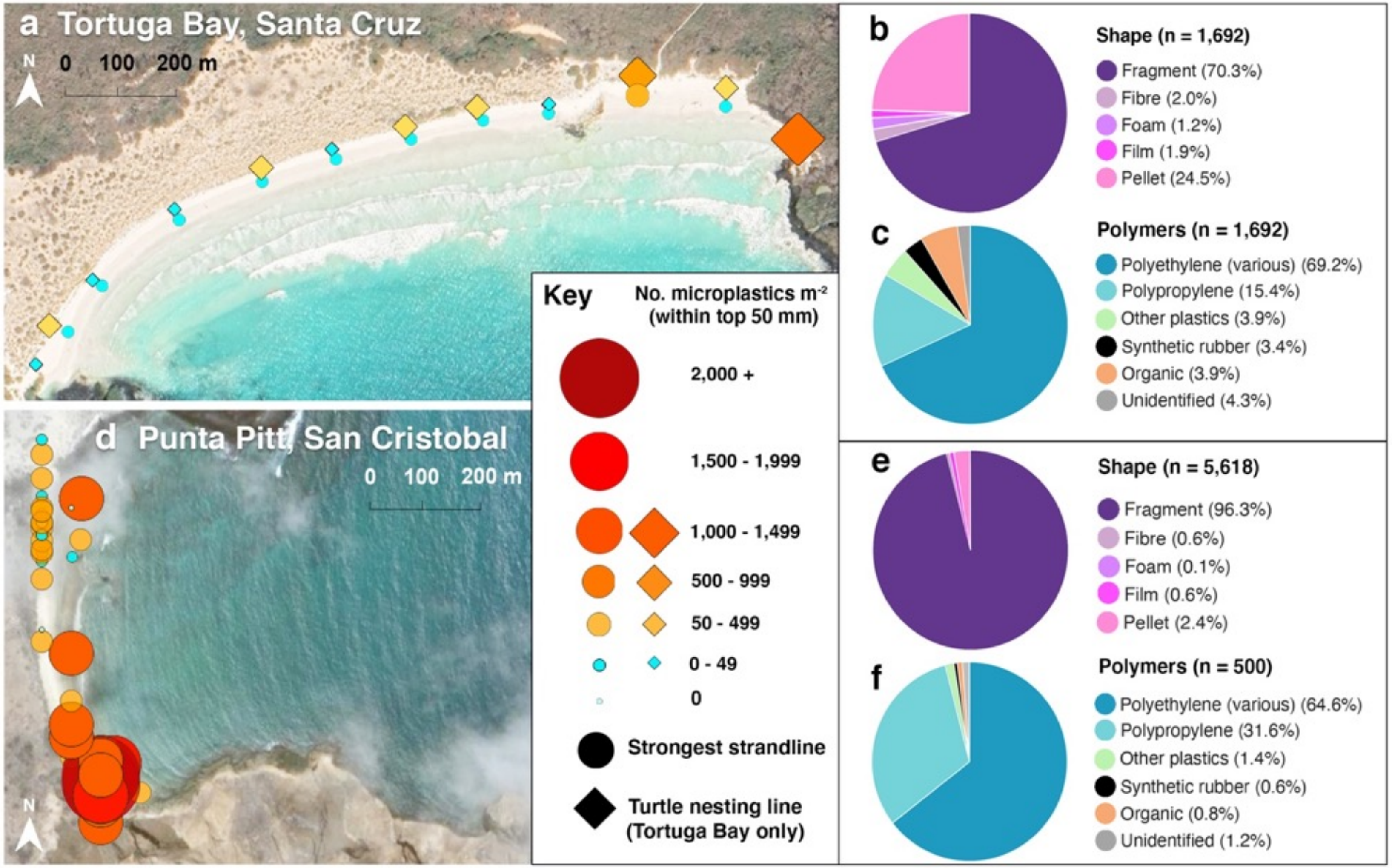

Figure 5.3: Citizen science survey results (April 2019 – January 2020). (a) Satellite image showing abundance recorded per m^2 at the heaviest strandline (circles) and at the turtle nesting line (diamonds) for Tortuga Bay, Santa Cruz showing approximate locations of 100 m between quadrats (n = 40), collected in March and August 2019, (b) shape composition and (c) polymer composition of particles from Tortuga Bay (n = 1,692), (d) Satellite image showing abundance recorded per m^2 in Punta Pitt, San Cristóbal island at the heaviest strandline (n = 54), collected between April 2019 and January 2020, (e) shape composition and (f) polymer composition of particles from Punta Pitt (n = 500). Satellite images retrieved from Google Earth Version 6.2. (accessed 18 February 2020), Galápagos Islands, http://www.earth.google.com).

Punta Pitt: Distribution and composition of beach microplastic

Overall, the mean number of microplastics at the strandline for Punta Pitt across all quadrats (381 ± 68 particles m^{-2} from 54 quadrats, April 2019 – January 2020) was much higher than Tortuga Bay (74 ± 43 particles m^{-2} from 20 quadrats, March 2019 – September 2019). Pooling the Punta Pitt data by transect (north vs. south quadrats), microplastic abundance was significantly higher on the southern side of the beach with a mean ± SD of 618 ± 104 particles m^{-2} compared to 125 ± 40 particles m^{-2} on the northern side (Wilcoxon Rank Sum test; W = 79, $p < 0.001$; Fig. 5.3d). Particles from Punta Pitt were mostly secondary fragments (96%, Fig. 5.3e) and 97% of the subset analysed by FTIR (n = 500) were polyethylene and polypropylene (Fig. 5.3f). Only 2% of particles from Punta Pitt were pellets.

When grouped by wet/ dry season, no clear seasonal patterns in microplastic abundance were evident in Punta Pitt (Wilcoxon Rank Sum Test, W = 9, $p = 0.9$) nor between months (Kruskall Wallis Test, H = 3.94, df = 8, $p = 0.86$) with both the highest and lowest concentrations recorded in the dry season. The difference between north and south grew more pronounced as the year progressed however, with low concentrations recorded in the north after the first two surveys (consistently < 200 particles m^{-2}, Fig. 5.4). The peak mean abundance was in July of 1,013 particles m^{-2} in the southern zone with the highest concentration in one quadrat measured as 2,524 particles m^{-2}.

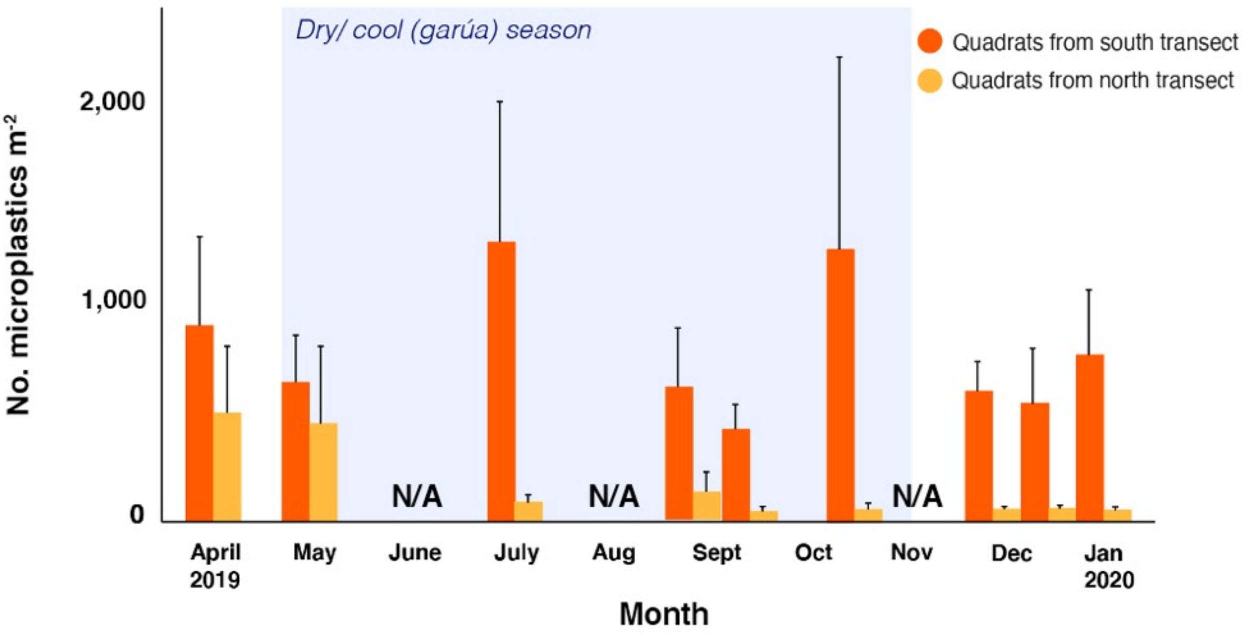

Figure 5.4: Microplastic abundance (particles m^{-2}) at Punta Pitt beach over the survey period (April 2019 – January 2020) across the south (orange bars) and north (yellow bars) zones of the. Error bars show standard error for quadrats for each survey. Surveys were not undertaken in June, August and November.

Surface characteristics and weathering of microplastics

Of the 2,192 particles categorised, 49% scored as extremely weathered and 34% as highly weathered (Fig. 5.5). The most weathered particles were polyethylene (66%) and polypropylene (22%) reflective of the composition of the entire sample, but natural particles identified as calcium carbonate (10%) also powdered easily under pressure and so generated a higher weathering score. A subsample of particles (n = 20) imaged by SEM showed a visual correlation of surface degradation with assigned weathering scores, particularly for pellets (Fig. 5.6); those scoring higher had increased cavities, flaking and fraying. The Punta Pitt subsample (n = 500) was also analysed for biofouling, present on 34 particles (7%), primarily suspected worm tubes or bryozoans. The most common particles to have visible biofouling were moderately weathered white polyethylene particles (65%, n = 22, mean weathering score = 3.1).

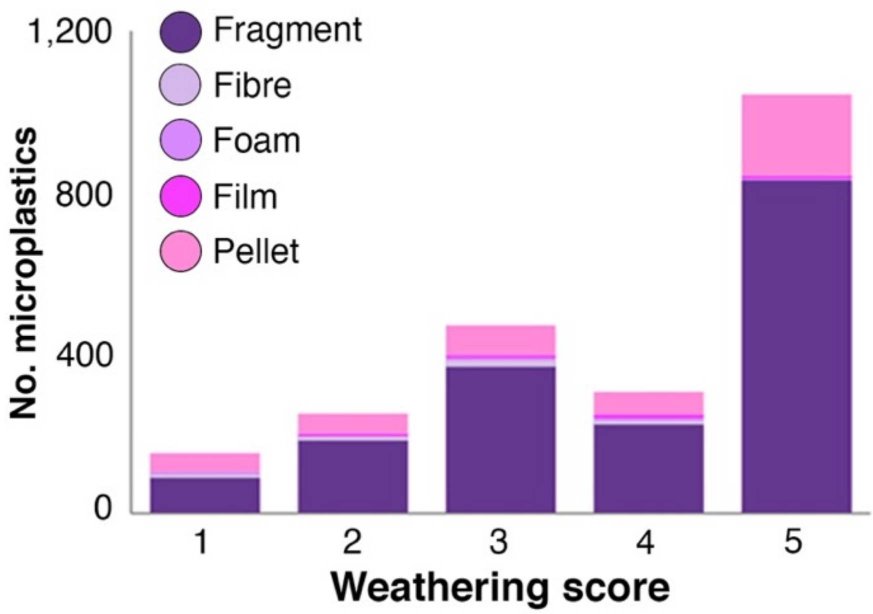

Figure 5.5: Barplot showing weathering scores across microplastic particle types (n = 2,192 from Tortuga Bay and Punta Pitt combined).

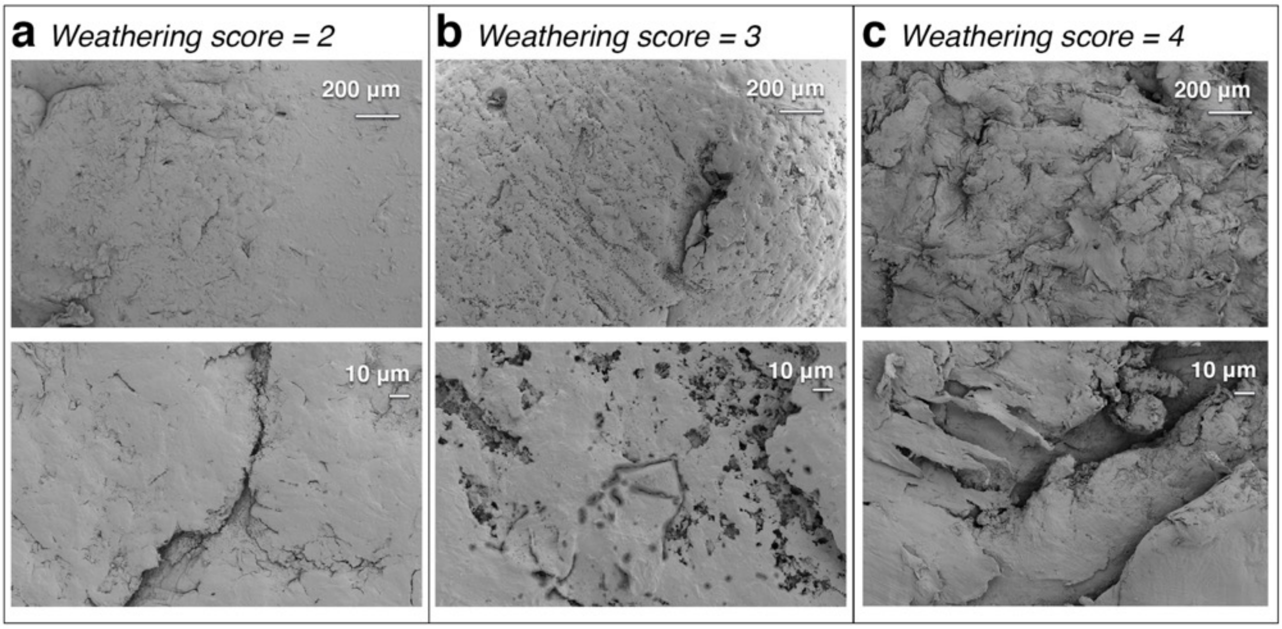

Figure 5.6: Scanning electron microscopy (SEM) images at 200 µm and 10 µm scale for three example polyethylene pellets collected from Tortuga Bay, weathering scores given by naked eye prior to imaging.

5.4. Discussion

Long term spatial and temporal datasets on microplastic contamination for any location are scare because they are difficult, time consuming and expensive to collect. This is especially true for remote islands where site accessibility is often an issue and potentially impacted species are of high conservation concern. Here we demonstrate the value of citizen science as an effective tool for collecting such data. Working with two student groups, we show microplastic identification for particles in the 1 – 5 mm size range is 93% accurate. Using a standardised sampling unit, this approach provided insight into spatiotemporal patterns of contamination for two sites of conservation interest in Galápagos revealing a strong accumulation gradient; perpendicular to the waterline in Tortuga Bay (up to four times higher in sea turtle nesting habitat compared to the strandline) and parallel to the waterline in Punta Pitt (five-fold higher concentrations in the south vs the north). Concentrations in excess of 2,500 particles m^{-2} were recorded in Punta Pitt in the dry season, although mean concentration across the beach during the whole survey was 347 particles m^{-2}, substantially lower than concentrations previously measured by citizen scientists in Rapa Nui (Easter Island) (805 particles m^{-2} in the top 20 mm of sand), probably due to its location in the Southern Pacific Gyre (Hidalgo-Ruz & Thiel, 2013).

Although validating crowd-sourced data is perhaps the most commonly cited barrier in citizen science (Follett & Strezov, 2015), only 19% of 120 marine citizen science studies recently reviewed employed validation strategies (Earp & Liconti, 2020), varying from low-tech options, such as the statistical comparison of researcher recounts (Hidalgo-Ruz & Thiel, 2013), to high-tech, specialised tools such as machine-learning algorithms for photographic data (Wolf *et al.*, 2020). As we were not able to perform laboratory recounts of sampled sand from quadrats, we are not able to validate the extraction efficiency, but all quadrats sampled in the field were checked by the trained coordinators. Survey coordinators were very important to increase confidence in results from citizen science data and to maximise educational opportunities for participants with complementary activities.

A key benefit of citizen science is the facilitation of inexpensive long-term data collection. Repeated sampling of the same beaches increases confidence that patterns observed are not errors from sampling design. Multi-year sampling would permit the investigation of seasonal variation, e.g. incorporating the effect of storm events that might influence beach microplastic abundance. This has been described in the Azores in the North-East Atlantic, where storms can cause major microplastic loading on sandy beaches following ejection from the gyre accumulation zones, with densities occasionally exceeding 15,000 particles m^{-2} (size range 2 – 5 mm) (Pham *et al.*, 2020). Whilst Galápagos is unlikely to experience input from the Pacific gyres, changes in the current regime such as during El Niño events may affect the volume and sources of plastic debris (Van Sebille *et al.*, 2019). The higher abundances of microplastics in the east-facing Punta Pitt beach compared to south-facing Tortuga Bay (overall mean 347 ± 178 particles m^{-2} vs 74 ± 43 particles m^{-2}) may be explained by its situation in the path of the prevailing Humboldt Current, previously postulated as a major source of plastic contamination to Galápagos (Van Sebille *et al.*, 2019). Also, the energy regime of each beach is likely to vary due to geophysical parameters, Punta Pitt is steeper which perhaps also affects deposition rates and the type of particles that are deposited. The difference between microplastic accumulation is less pronounced between the east and western zones of Tortuga Bay suggesting that fine-scale currents are perhaps less influential at this site than at Punta Pitt.

Before this project began, Punta Pitt was not cleaned meaning that plastic debris could accumulate and breakdown *in situ* generating microplastics and adding to the standing stock. In contrast, macroplastic is cleaned on an almost daily basis in Tortuga Bay suggesting that microplastics are more likely to arrive already in smaller fragments at this site. This suggests that clean ups are likely to be effective in decreasing overall microplastic concentrations however, also due to the possibility that these fragments washing up in Tortuga Bay are potentially from sources within the Archipelago as well as from outside the marine reserve. The high incidence of industrial pellets in Tortuga Bay, even immediately following a major pellet clean-up, identifies the plastic manufacturing industry as a source of primary microplastics to the Galápagos Marine Reserve. The closest plastic manufacturing facility is in the city of Guayaquil in mainland Ecuador, > 1,000 km to the east but oceanographic modelling has shown that the arrival of floating microplastics within several months is possible from continental Ecuador (Van Sebille *et al.*, 2019). At sea spillages may also be a potential source. Pellets showed variable weathering suggesting that they are from a series of leak events over time. Over 90% were found at the turtle nesting line, indicating that pellets are transported up the beach and being sequestered into the surface layer of sediment, perhaps more likely than fragments or fibres due to their rounded shape.

Our laboratory analysis demonstrates the potential of by-eye particle weathering assessment as a useful indicator for the age of beach microplastic linking to possible contamination sources. Most sampled microplastics were highly weathered suggesting that they have been in the environment for long timescales, possibly in the order of decades. The physicochemical properties of plastic polymers and their additives can affect the rate of weathering (as reviewed by Liu *et al.*, 2020) although this is not always predictable as the changes to chemical bonds are not necessarily linear over time, suggesting this information should perhaps be used cautiously (Brandon *et al.*, 2016). The monitoring of rafting species on plastics is especially important for Galápagos with the threat of non-native species invasions to the sensitive ecosystem (Keith *et al.*, 2016). It is also important for understanding plastic movement in the environment; biofouling may act as a signpost for the journey of a piece of plastic but also affects the sinking velocity of particles, causing low density polymers such as polyethylene to sink (Kaiser *et al.*, 2017). In our study, biofouling was most common on polyethylene fragments that were moderately weathered, primarily comprised of desiccated worm tubes and bryozoan mats. Settling dynamics of rafting species on microplastics is not well understood, although it is assumed that the surface area would affect what organisms are able to attach suggesting that larger fragments would be more likely to be colonised (Fazey & Ryan, 2016a). The uneven surface of heavily weathered particles may not be optimum for biofouling by larger organisms although the increased surface area caused by cracks and crevices may be favourable for increased colonisation by micro-organisms. These methods need to be tested with citizen scientists but represent a potential route to add value to data collection in the future.

The high sorption capacity for POPs and pathogens make microplastics potential passive environmental samplers but also represent an ecotoxicological risk for ingesting fauna (Floren & Shugart, 2017; Pozo *et al.*, 2020). The International Pellet Watch reported light PCB pollution (12.0 ng/g) in pellets from Tortuga Bay collected in 2014 by citizen scientists, although concentrations as high as 90.0 ng/g were recorded suggesting that ongoing POPs monitoring should be a priority, particularly when considering the vulnerability of native wildlife to chemical stressors (Alava *et al.*, 2014; Karapanagioti & Takada, 2019). Other potential wildlife impacts may originate from the incorporation of microplastics into sediments that could increase permeability leading to desiccation stress for meiofauna and decrease temperatures (Carson *et al.*, 2011) altering nesting dynamics of marine reptiles such as sea turtles and marine iguanas. In Tortuga Bay, microplastic abundance increases towards the back of the beach where turtles nest, suggesting that for wind exposed, dissipative beaches, microplastic accumulation hotspots are not necessarily at the strandline, a consideration for future study design.

To fully understand the plastic budget at an Archipelago scale, comprehensive scientific sampling campaigns, including multiple habitats and beach types, seasonal variation and ecological monitoring are required. Citizen science can support this, filling the temporal gaps at selected sites, supporting management and engaging both community members and tourists. The heavy protection of Galápagos is an understandable barrier to accessibility and scaling up citizen science initiatives, with most beaches having either no public access or access granted only to small, scheduled tourist groups. National Park Guides could provide a network to monitor microplastics at an Archipelago scale if kits (e.g. quadrat, sieves, bucket and data sheet/ photo guide) and training were provided as they have regular access to a wide range of sites of high conservation relevance. Plastic collected could be pooled or kept in paper envelopes for future data validation via recounts and FTIR analysis.

For future work, we recommend a flexible approach to sampling design to allow for the tailoring of projects to support positive experiences for participants considering feasible time constraints, skill level and attention spans (Kobori *et al.*, 2016). Previous work has shown that the easier data is to collect, the better accuracy tends to be (Parsons *et al.*, 2011). Sieving can be laborious (especially in coarser sediment sites) and so this method is unlikely to be suitable for younger citizen scientists unless it is modified to be quicker, perhaps facilitated by smaller quadrats (in area, maintaining the consistent 50 mm depth for comparability). Many managers and researchers monitoring plastic in remote areas do not have easy access to spectroscopy equipment such as FTIR to verify polymer type of suspected microplastics. Following an initial survey where a percentage of particles are analysed by spectroscopy, the application of a conversion factor (such as our 93%) to citizen generated data could control for misidentified particles, negating the need for more than a small sub-sample to be analysed periodically via spectroscopic methods.

Engaging citizen scientists in our age bracket (15 – 25 years) in the development of additional hypotheses for surveys and in data analysis, provided an additional layer of engagement; the use of the standardised sampling unit ensured that data were comparable, but students could follow their own lines of enquiry to give the surveys a research question within their own right. The benefit of engaging citizen scientists above solely data collection has been shown in an Australian phenology citizen science project *ClimateWatch*, where undergraduate students showed an improvement in data collection quality when tasked with analysing existing data as well as an increase in environmental engagement, defined as ongoing non-mandatory involvement in the programme or reporting an increased interest and connection with nature (Mitchell *et al.*, 2017). Dean *et al.* identify three key indicators for greater environmental engagement: the willingness to share information (our students pro-actively presented their findings at community events several times over the project), increased support for conservation or citizen science (many volunteered for repeated surveys) and the intentions to change behaviour (we did not measure this, but would like to explore the drivers for this in different audiences in future work) (Dean *et al.*, 2018). Finally, working with NGOs and National Park authorities helps to ease project management burden on researchers, provides access to long term monitoring programmes and data repositories and supports quicker and more likely translation to management action and therefore, is highly recommended for future studies (Rambonnet *et al.*, 2019). The installation of effective locally ran monitoring schemes not only strengthens capacity but reduces traffic to sensitive systems by scientists and reduces the carbon footprint of global travel.

5.5. Conclusion

The results of this study show that reliable beach microplastic monitoring data can be collected by student citizen scientists, presenting a valuable addition to ongoing monitoring efforts. By using the standard unit of a quadrat excavated to a known depth at the strongest strandline, we can compare sampling over time, supporting extrapolation of trends. This in turn can help to identify sources, identify accumulation zones to establish ecological risks and design solutions. We show a variety of sources of microplastic pollution to Galápagos, including floating polyethylene or polypropylene hard fragments and industrial pellets, highlighting that action is needed along the entire plastics chain, from design and manufacture to end-of-life and waste management. As part of a multi-institutional initiative, policymaker engagement was incorporated in early design phases of this project echoing the benefits of interdisciplinary collaborations highlighted in a review by Rambonnet *et al.* (2019). A coordinated approach at a regional scale in the Eastern Pacific to beach plastics monitoring could fill major knowledge gaps and if established over the long term, could compensate for the climatic complexity and variability in this understudied region (Wood *et al.*, 2016).

5.6. Acknowledgements

We acknowledge the hard work of the student citizen scientists and educators who made this study possible particularly María Luisa Buitron, Kevin Cabrera, Ana Belen Jaramillo, Pablo Jaramillo, Lady Marquez, Marlon Mora, Anais Suntaxi, Henry Vivanco, Fiorella Vizuete, the Mola Mola Club and the Marine Reserve Student Participation Project. We would like to thank the administrative staff from the Galápagos Science Center for facilitating logistics, Manuel Yépez from SharkSky Travel & Conservation for his generous donation of boat time to support the Punta Pitt fieldwork and the Galápagos National Park Directorate for the trust granted to undertake this work. Thanks also to Dr Christian Hacker for supporting SEM analysis. This study is part of the 'Plastic Pollution Free Galápagos' initiative and falls under the "*Understanding the Effects of Marine Debris in the Galápagos*" permit authorised by the Galápagos National Park Directorate (GNPD) (PC-23-19). This research was funded by grants received by JJ from the Galápagos Conservation Trust, the Woodspring Trust and the British Embassy Quito and individual donors to the University of Exeter received by CL. This is a pilot project for the Global Challenges Research Fund (GCRF) grant (NE/V005448/1).

5.7. Supplementary Materials

Supplementary Table 5.1: Weathering scoring criteria for particles (1 – 5 mm).

Weathering Score	***Description***
1	Smooth surface, no visible scratches, cracks or biofouling
2	Evidence of light weathering (scratches, shallow cracks, biofouled), no discolouration or yellowing
3	Visually weathered (uneven surface, frayed edges, deep cracks, biofouled, yellowing or discoloured), withheld pressure of ATR probe
4	Highly weathered (sub-surface level cracks, frayed edges, biofouled, yellowing or discoloured, brittle), cracked under pressure of ATR probe
5	Extreme weathering (sub-surface level cracks, frayed edges, biofouled, yellowing or discoloured, brittle), powdered under pressure of ATR probe

Supplementary Figure 5.1: Photographs of Tortuga Bay, Santa Cruz, Galápagos Islands, Ecuador. (a) Looking east along the beach, (b) looking west along the beach (credit: Adam Porter).

Supplementary Figure 5.2: Photographs of Punta Pitt beach, San Cristóbal, Galápagos Islands, Ecuador. (a) Looking north along the beach, (b) looking south along the beach (credit: Adam Porter).

Supplementary Table 5.2: Playas sin Plásticos – Datasheet (translated to English).

<table>
<tr><th colspan="7">MONITORING INFORMATION (please be as precise as possible)</th></tr>
<tr><td>LOCATION</td><td colspan="3">Island:</td><td colspan="3">Site name:</td></tr>
<tr><td>DATE & TIME</td><td colspan="2">Monitoring date:</td><td colspan="2">Start time:</td><td colspan="2">Start end:</td></tr>
<tr><td>PARTICIPANTS</td><td colspan="3">No. of participants:</td><td colspan="3">Name of data manager:</td></tr>
<tr><td rowspan="2">BEACH</td><td colspan="4">Type of beach:
(fine sand/ gravel/ pebbles)
Note the colour of sediment</td><td colspan="2">Main use of beach:
(tourists/locals/fishing)</td></tr>
<tr><td colspan="4">Is this beach cleaned?: Yes / No / I don't know
If it's cleaned…
- Who does it?
- How often?</td><td colspan="2">Slope of the beach:
(0% = flat
100% = vertical)</td></tr>
<tr><td>TIDE</td><td colspan="2">Coefficient:</td><td colspan="2">Time of high tide:</td><td colspan="2">Time of low tide:</td></tr>
<tr><td>WEATHER</td><td>Wind:
none / light breeze / moderate / strong</td><td colspan="2">Wind direction:
N – NE - E – SE –S – SW –W – NW</td><td>Cloud cover:
(What % of sky is covered in clouds?)</td><td>Rain:
During monitoring:
Yes / No
During the night before:
Yes / No</td><td>Temperature:
_______º

(in degrees centigrade)</td></tr>
<tr><td>COORDINATES GPS: TRANSECT 1 (50 metres)</td><td>GPS Coordinates start of transect</td><td>S: 00. ____________
W: 090. ___________</td><td colspan="2">GPS Coordinates end of transect</td><td colspan="2">S: 00. ____________
W: 090. ___________</td></tr>
<tr><td>COORDINATES GPS: TRANSECT 2 (50 metres)</td><td>GPS Coordinates start of transect</td><td>S: 00. ____________
W: 090. ___________</td><td colspan="2">GPS Coordinates end of transect</td><td colspan="2">S: 00. ____________
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<tr><td>QUADRATS (50 cm x 50 cm x 5 cm)</td><td>GPS Coordinates of Quadrat A (Transect 1)</td><td>S: 00. ____________
W: 090. ___________</td><td colspan="2">GPS Coordinates of Quadrat D (Transect 2)</td><td colspan="2">S: 00. ____________
W: 090. ___________</td></tr>
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W: 090. ___________</td><td colspan="2">GPS Coordinates of Quadrat E (Transect 2)</td><td colspan="2">S: 00. ____________
W: 090. ___________</td></tr>
<tr><td></td><td>GPS Coordinates of Quadrat C (Transect 1)</td><td>S: 00. ____________
W: 090. ___________</td><td colspan="2">GPS Coordinates of Quadrat F (Transect 2)</td><td colspan="2">S: 00. ____________
W: 090. ___________</td></tr>
<tr><td>PHOTOS</td><td colspan="6">How many photos were taken? On which camera or phone?</td></tr>
<tr><th colspan="7">OBSERVATIONS DURING MONITORING</th></tr>
<tr><td colspan="7">Number of people/ boats observed

Wildlife observed:

Other observations:</td></tr>
</table>

Chapter 6

General Discussion

The increase in marine plastic contamination, a tangible result of global consumerism, has raised concerns for ecologically vulnerable ecosystems such as in the Galápagos Islands, Ecuador. In this thesis, I have: (i) described high variation in spatiotemporal trends in plastic contamination across the Galápagos Marine Reserve, identifying hotspot accumulation zones (**Chapter 2**, **Chapter 4** & **Chapter 5**), (ii) observed microplastic uptake in seven species of marine invertebrate (**Chapter 2**), (iii) developed a priority scoring analysis to investigate risk of harm across the marine food web (**Chapter 2** & **Chapter 3**) and (iv) tested citizen science methods to support the scaling of cost-effective monitoring of macroplastics and microplastics (**Chapter 5**). In this discussion, I explore how my research contributes to our understanding of plastic pathways and fates in the environment, how potential risks to marine ecosystems might interact with multiple stressors and make some recommendations for future research priorities for Galápagos to fill remaining knowledge gaps.

Spatiotemporal trends in marine plastic contamination

The identification of plastic accumulation zones and temporal spikes in concentrations is important to understand environmental pathways and fates, to highlight potential risks to biota and to support the development of mitigation actions (Vince and Stoett, 2018; Nel *et al.*, 2020; Nelms *et al.*, 2020). My research has shown that the distribution of macroplastic and microplastic pollution is highly variable at a variety of resolutions in the Galápagos Marine Reserve; at an Archipelago scale (microplastics were six to ten-fold higher at the seawater surface, in benthic sediment and in beach sand in the populated South-Central Zone compared to the upwelling Western Zone, **Chapter 4**), at an island scale (large beach microplastics were significantly higher on east-facing bays (mean ± SE 622 ± 108 particles m^{-2} versus island mean of 53 ± 24 particles m^{-2}) and microplastics were significantly higher at the seawater surface closer to human populations (0.89 particles m^{-3} versus an island mean of 0.16 particles m^{-3}, **Chapter 2**) and at a beach (microplastic concentrations were > 300% higher in the south of the beach compared to the north at Punta Pitt (618 ± 104 particles m^{-2} versus 125 ± 40 particles m^{-2}) and > 400% higher at the turtle nesting line compared to the strandline at Tortuga Bay (440 ± 167 particles m^{-2} versus 95 ± 56 particles m^{-2}), **Chapter 5**)). The future description of ecological communities at these hotspot sites that harbour consistently higher concentrations will be important to delineate what species might be interacting in these areas where encounter rate is likely to be higher.

These data add to growing global evidence that microplastics in oceanic waters vary considerably in time and space. This has been described in the Eastern Pacific via analysis of an 11 year surface tow dataset where large-scale surface currents and wind variability are the primary drivers of the variation in surface microplastic concentration, showing differences of two orders of magnitude spatially (within 32 km; 1.232 x 10^{7} pieces km^{-1} versus 2.038 x 10^{4} km^{-1}) and inter-annually (two orders of magnitude increase between 1999 and 2010 in the same area) (Law *et al.*, 2014). At a multi-annual scale, ENSO and the Pacific Decadal Oscillation modify oceanographic processes in the Eastern Pacific including mesoscale eddies and sub-mesoscale processes such as upwelling and vertical mixing, all drivers of plastic movement (Van Sebille *et al.*, 2020). Currently, the volume of plastic modelled to enter the Galápagos Marine Reserve under ENSO scenarios is not expected to be significantly different although it will likely originate from more northerly sources, potentially changing the composition profile (Van Sebille *et al.*, 2019). Seasonal changes at a shorter time-scale also affect the distribution and composition of microplastics in the beach environment with increased rainfall, flooding and storm events often increasing deposition from marine sources (Balthazar-Silva *et al.*, 2020; Pham *et al.*, 2020; Veerasingam, Mugilarasan, *et al.*, 2016), increasing run off from terrestrial sources (Piñon-Colin *et al.*, 2020) and also potentially removing plastics from sediments via scouring action and resuspension processes (Horton & Dixon, 2018).

Due to the inherent interconnectedness of the natural and built environment, the pathways and fates of plastics cannot be fully understood by considering a single habitat or via a single discipline (Hale *et al*., 2020). As the field evolves from the historical focus of sampling floating plastics in surface ocean waters, efforts are growing to sample other habitats such as deep sea waters (Courtene-Jones *et al.*, 2017), deep sea sediments (Barrett *et al.*, 2020; Fischer *et al*., 2015), within beach sediment (Turra *et al.*, 2014), freshwaters (Miller *et al.*, 2017; Donoso and Rios-Touma, 2020), soils (Rillig, 2012; Prata *et al.*, 2021) and the air (Stanton *et al.*, 2019), growing our collective knowledge of how plastics move around the environment. Zhu (2021) has recently proposed that we might consider this 'plastic cycle' as a branch of the carbon cycle, due to the fact that plastics represent a substantial store of carbon and move within environments potentially analogously to carbon fluxes (Zhu, 2021). This constant movement also means that one-off environmental measurements are unlikely to capture a reliable estimate of plastic stocks and fluxes, again suggesting that larger spatiotemporal datasets are increasingly important to progress our understanding. Possible benefits of adopting this well-known framework would be a shared terminology (e.g. fluxes, reservoirs and sinks), the promotion of a joined-up view between studies and perhaps support for knowledge transfer between disciplines.

Understudied coastal sites that may be plastic sinks

In order to understand the true standing stock and fluxes of plastics, the full spectrum of coastline habitats and beach types must be considered. Galápagos beaches are highly variable, impacted by tectonic activity and lava flows, the extent of fringing mangrove systems and the characteristics of local ecology that impact sediment dynamics (Walsh *et al.*, 2016). 'Coral rubble' beaches such as those at Rosa Blanca on the east coast of San Cristóbal, are a result of storms breaking off and depositing dead corals along the coastline, likely bleached during the major ENSO of 1983 that the majority of Galápagos reefs have still not recovered from (Glynn *et al.*, 2018). Initial survey of just a 10 m^2 area (5 m x 2 m transect excavated to an estimated 5 – 10 cm depth), yielded a count of 1,168 macroplastic items (116.8 items m^{-2}), far exceeding the maximum density on sandy beaches (0.66 items m^{-2} in Puerto Tablas, San Cristóbal). This was made up of mainly plastic film/ sheeting (42.6%) and ropes (32.4%), with only a small percentage of fragments (11.9%) (Fig. 6.1 a-b), a very different profile of plastic composition to sandy beaches. Fragments are perhaps more likely to be washed away on the tide as opposed to films and ropes that become trapped in the irregular sediment. No published information was found on the ecology of these coral rubble beaches meaning the potential risks of these accumulations are unknown.

Another potential plastic sink in a habitat we do know to be very ecologically important, is mangroves. Although previously neglected due to the challenges of sampling and access, the characterisation of plastic pollution impacts in mangroves is an emerging area of research (Martin *et al.*, 2019; Govender *et al.*, 2020; Li *et al.*, 2020). Mechanical snagging of macroplastic and retention of microplastics in mangrove sediments demonstrate their potential role in modulating plastic pathways and fates (Naji *et al.*, 2019). In Galápagos, mangroves represent important nursery sites for many marine vertebrates including threatened elasmobranchs such as the scalloped hammerhead (*Sphyrna lewini*) (Chiriboga *et al.*, 2021; in press), commercially important fishery species and shorebirds such as the lava gull (*Leucophaeus fuliginosus*) that scored highly in the priority analysis (**Chapter 2**). Mangroves cover 35% of coastline in Galápagos, in both exposed and sheltered bays and plastic accumulations were observed during fieldwork (Fig. 6.1 c-d), mostly retained behind the mangrove line, probably deposited during storm events. The quantification of these plastic sinks, their composition and the delineation of potential risks to mangrove-dwelling species are recommended as a future research priority.

Figure 6.1: Photographs of understudied coastal plastic sinks. (a) Coral rubble beach at Rosa Blanca on the east coast of San Cristóbal; (b) composition of sub surface plastics on a coral rubble beach including primarily fragmented films and ropes; (c) macroplastic accumulation behind a mangrove forest patch on the east coast of San Cristóbal; (d) plastic integrated in mangrove leaf litter at the same site. (a-b) credit: Jen Jones, (c-d) credit: Adam Porter.

***In situ* weathering and fragmentation of plastics in the beach environment**

Over 90% of microplastics recorded in this study are from secondary sources (i.e. a result of the degradation and fragmentation of other items). This is typical for the majority of global environmental sampling (as reviewed by Burns & Boxall (2018) using 109 studies), as well as the most commonly found particles in seafood species (as reviewed by Santillo *et al.*, 2017). There are many variables that affect weathering and fragmentation including the material characteristics of plastics, such as particle density (affecting how particles partition in the environment and what weathering processes they are subjected to), partial crystallinity (affecting susceptibility to oxidative degradation), the degree of elasticity (affecting how brittle plastics are and how susceptible they are to tensile tearing) and surface properties (affecting the rate of fouling, linked with biological weathering and sinking) (Andrady, 2017; Efimova *et al.*, 2018). Song *et al.*, demonstrated the variable weathering behaviours of different polymers by exposing polyethylene and propylene pellets to UV irradiation for a year and then subjecting them to mechanical abrasion by sand for two months to simulate weathering in the beach environment. This produced 20 ± 8 particles per polyethylene pellet and 6,084 ± 1,061 particles per polypropylene pellet, demonstrating that polypropylene is much more susceptible to weathering (Song *et al.*, 2017). This was also seen during the visual weathering assessment of particles in **Chapter 5** where weathered polypropylene particles had much more degraded surfaces and often completely powdered under the pressure of the FTIR probe. This suggests that different polymers may pose different risks to biota due to this faster degradation with more, smaller-sized particles, increasing bioavailability (Wright *et al.*, 2013).

Probable *in situ* fragmentation was much easier to observe on lava rubble beaches (Fig. 6.2a) than in the dynamic environment of sandy beaches where plastic debris is often washed in and out by the tide and blown around by the wind. In the west of Isabela island during sampling for **Chapter 4**, I observed large blue and green polypropylene ropes that had presumably degraded *in situ*, surrounded by thousands of smaller fibres (Fig. 6.2b-c), verified by FTIR analysis of 50 fibres from each, all returning the same spectra matches for polypropylene. In addition to probable evidence of *in situ* fragmentation, incidences of plastics melted onto rock surfaces forming 'plasticrusts' (Fig. 6.1d) were observed, in addition to 'plastiglomerates', where plastics and rock fragments had melded together (Fig. 6.2e), both geological:plastic interactions originally described in the volcanic Archipelago of Hawaii (Corcoran *et al.*, 2014; Gestoso *et al.*, 2019). This suggests that in addition to considering degradation and fragmentation processes in the plastic cycle for volcanic islands such as Galápagos, melting and the formation of aggregations should also be factored into mass balance models.

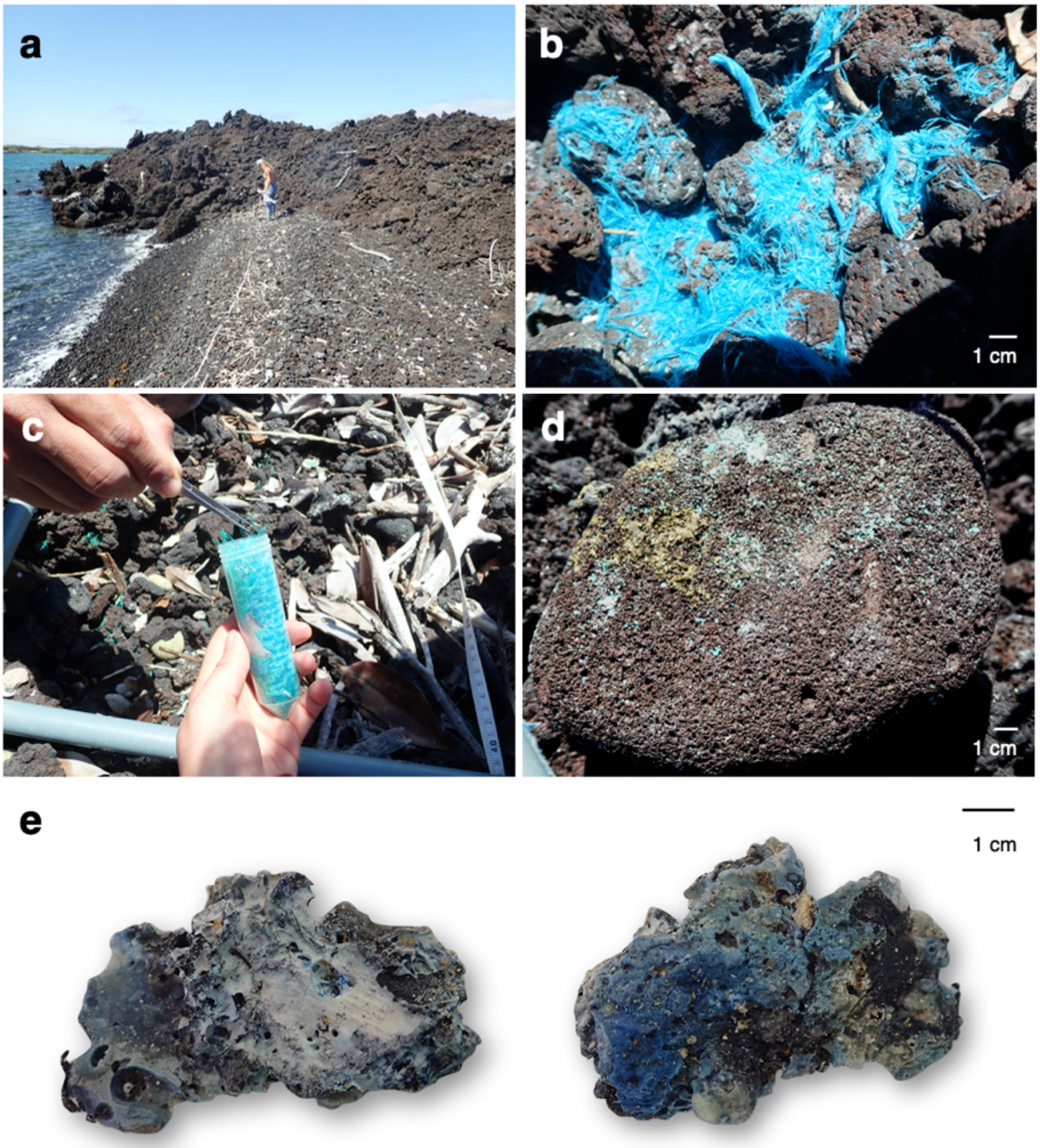

Figure 6.2: Photographs of plastics on lava rubble beaches. (a) A typical lava rubble beach on western Isabela island, (b) a fragmented blue polypropylene rope, (c) attempting to collect polypropylene microfibres from a quadrat, likely from the same original green polypropylene rope (as verified by FTIR polymer analysis), (d) an example of a 'plasticrust' forming, melted into the crevices of rock and e) an example of either side of a 'plastiglomerate' (plastic/ rock compound) that contained traces of both polyethylene and polypropylene.

Methods development: Monitoring beach plastics via Citizen Science

A coordinated approach to data collection at a regional scale would fill geographic knowledge gaps and, if established over time, could provide a time-series, compensating for the complexity and variability in the region (Wood *et al.*, 2016). In **Chapter 5**, I explored the potential of citizen science monitoring for large microplastics (1 – 5 mm) on sandy beaches in Galápagos, using a standard quadrat (50 cm x 50 cm x 50 mm) method to sieve surface beach sediment. This project was designed following a detailed literature search to understand key barriers in previous beach plastic citizen science projects, particularly for microplastics. I followed the recommendations of Rambonnet *et al.* (2019), generated from qualitative interviews with ten marine plastic citizen scientist project leaders including getting local authorities and community groups involved early in project design to ensure buy-in, recruiting and training consistent survey coordinators and co-designing supplementary activities to enhance the potential educational benefits for participants (Rambonnet *et al.*, 2019).

Student citizen scientists (aged 15 – 24 years) in our study were 93% accurate at visually identifying plastic and synthetic rubber particles, as verified by FTIR analysis of polymers of c.30% of the total sample. This method therefore presents a way to characterise spatiotemporal variation of beach microplastics without the need for access to expensive spectroscopy equipment, potentially very useful for expanding this project to the wider region. The ongoing involvement of school students and eco clubs is continuing into 2021 through partnerships with the Galápagos Conservation Trust, Galápagos Science Center and Galápagos National Park Directorate and is planned to be a long-term activity as part of the ongoing "Playas Sin Plásticos" programme. In addition to the scientific and management utility of these long-term datasets, there are potential wider benefits of citizen science from an educational and environmental engagement perspective. Opportunities to collaborate between disciplines with social scientists to understand more about environmental attitudes and linking with sustainable behaviours would be an interesting way to develop this work in the future.

Together with the beach microplastic sampling method, I developed a macroplastic survey method building on that described in **Chapter 2** i.e. the collection and categorisation of all surface macroplastic items (> 5 mm) from two representative transects (50 m length, with all items collected from waterline to vegetation line). Categorisation need not happen in the field or even in Galápagos if samples are photographed, representing further potential for digital citizen science as demonstrated in **Chapter 4 & 5** and via a successful undergraduate dissertation project that used macroplastic photographs to remotely assess spatiotemporal trends. This method is now being used in annual monitoring surveys conducted by the Galápagos National Park Directorate including during a rapid assessment survey of visitor sites during the COVID-19 closures (June – December 2020) to investigate if tourism presence actually has a positive effect on plastic contamination control in Galápagos i.e. through increased cleaning. As this method is surface collection over a known area (resulting in a metric of items m^{-2}) and by photographing the sample after collection, this method could be modified for other habitats such as rocky shores and terrestrial environments and is easily comparable with other studies around the world.

Prioritisation Scoring as a rapid assessment tool

Translating evidence of microplastic uptake as observed in Galápagos marine invertebrates, to the prediction of potential harm, is a major challenge in the field. In **Chapter 3**, I explore the limits of using bioindicator species to monitor microplastics from a physiological perspective (i.e. that retention is not generally predictable or necessarily cumulative in organisms) and the ethical perspective in terms of sacrificial sampling of organisms in a protected area. There is increasing interest in the field to embrace non-lethal sampling methods using linked health indicators e.g. physiological parameters or histopathology (Puskic *et al.*, 2020), such as the relationship described between muscle atrophy and lack of subcutaneous fat and the mass of plastic ingested by the seabird, the Northern Fulmar (*Fulmarus glacialis*) (Donnelly-Greenan *et al.*, 2014). This has also recently been attempted for invertebrates; using solid phase microextraction coupled with liquid chromatography mass spectrometry analysis, showing that phthalate esters (plasticizers) were measurable in a scleractinian coral (*Danafungia scruposa*) and a bivalve mollusc (*Tridacna maxima*). A small amount of tissue damage occurred during sample extraction but nevertheless, this may represent a preferable sampling method to avoid the sacrifice of organisms, particularly if investigating threatened species such as those in Galápagos (Saliu *et al.*, 2020). The type of sampling method will depend on the research question at hand however, and although these techniques may provide presence/absence data, there is not yet a reliable way to quantify ingested particles or to investigate the shape or polymer ingested via non-lethal methods.

To provide an alternative tool based on existing data, a priority scoring methodology was developed (**Chapter 2** & **Chapter 3**). This tool will benefit substantially from improved field-based evidence of wildlife-plastic interactions in the region and environmental contamination across relevant habitats. This method has already inspired a similar tool for the Ganges river system which in addition to the species distribution, conservation status and literature evidence scoring used in this study, also incorporated a score for discarded plastic fishing gear density based on field data. Similarly to the Galápagos system, literature evidence of negative interactions for the Ganges species assessed was severely lacking and generally absent, although this knowledge gap was partially addressed via interviews with fishers, demonstrating the value of interdisciplinary approaches (Nelms *et al.*, 2021). Recently, citizen scientists reporting vertebrate entanglement via social media have also proved an effective data collection mechanism to add to the knowledge base for local wildlife-plastic interactions (Donnelly-Greenan, Nevins & Harvey, 2019; Parton *et al.*, 2019) and a study for Galápagos has recently been launched by our close collaborator Juan Pablo Muñoz-Pérez to address this knowledge gap. With the combination of observations of interactions with native species together with the environmental baseline presented in this thesis, the priority scoring analysis could be further improved to direct mitigation action and future plastics research in Galápagos. There are also plans to apply this tool to the wider Eastern Pacific Region to provide a prioritisation assessment for wider marine biodiversity in this ecologically and socioeconomically important region.

Multi-stressor effects

In any marine ecosystem, plastic pollution is being experienced by organisms against a background of other stressors including sea surface warming, acidification, severe overexploitation of natural capital and chronic pollution (Abessa *et al.*, 2018; IPCC, 2019). Galápagos is subject to strong effects from ENSO events when water temperature may be 10 – 18°C above the normal range of the Humboldt Current (Wingfield *et al.*, 2018) introducing considerable temperature shock as well as food limitation from the impeded primary productivity. Entire ecological regime shifts have followed ENSO events with coral and macroalgal habitats replaced by heavily grazed reefs dominated by crustose coralline algae making 'urchin barrens', a shift likely magnified by the removal of predators such as spiny lobsters by elevated fishing effort (Edgar *et al.*, 2010). This has had major effects for range-restricted vertebrates such as the endemic Galápagos penguin (*Spheniscus mendiculus*), flightless cormorant (*Phalacrocorax harrisi*) and marine iguana (*Amblyrhynchus cristatus*) that saw drastic declines of 77%, 49% and 70% respectively after the 1983 ENSO event (Valle *et al.*, 1987; Laurie, 1990). The afore-mentioned vertebrates are all high scoring in the priority scoring analysis raising concerns over the added pressure of increasing plastic contamination.

Key pathways of marine pollutants in Galápagos with a summary of potential impacts to health and fitness of marine species are demonstrated in Fig. 6.3 and Fig. 6.4. It is not well understood how different stressors interact and what subsequent health impacts organisms might be subjected to, partly due to the challenges of studying multiple stressors in exposure experiments due to the plethora of potential combinations and how quickly that can become unfeasible to control and monitor. A review by Sokolova (2021) highlights the potential impacts of multiple stressors on organism bioenergetics, including adverse effects on cellular processes such as detoxification mechanisms, to possible ecological consequences of population level effects from depressed metabolic rates (Sokolova, 2021). As plastic ingestion has been linked with effects such as satiation and compromised ability to take up nutrients from the diet (Cole *et al.*, 2015; Matheus *et al.*, 2020), there is a chance that microplastics may leave organisms energetically compromised to deal with other stressors. Wider ecosystem effects may also be more likely during this time when ecosystem functioning is compromised, perhaps leaving communities more vulnerable to non-native invasions. Although tolerance to heat stress and food limitation may be selected for in Galápagos species due to high environmental variation, there is no evidence for how species might be affected by a dynamic, heterogenous novel contaminant such as plastic pollution. Reduced species diversity of endemic functional groups is a potential long-term effect if plastic impact is disproportional across geographic and trophic gradients (Alava *et al.*, 2020).

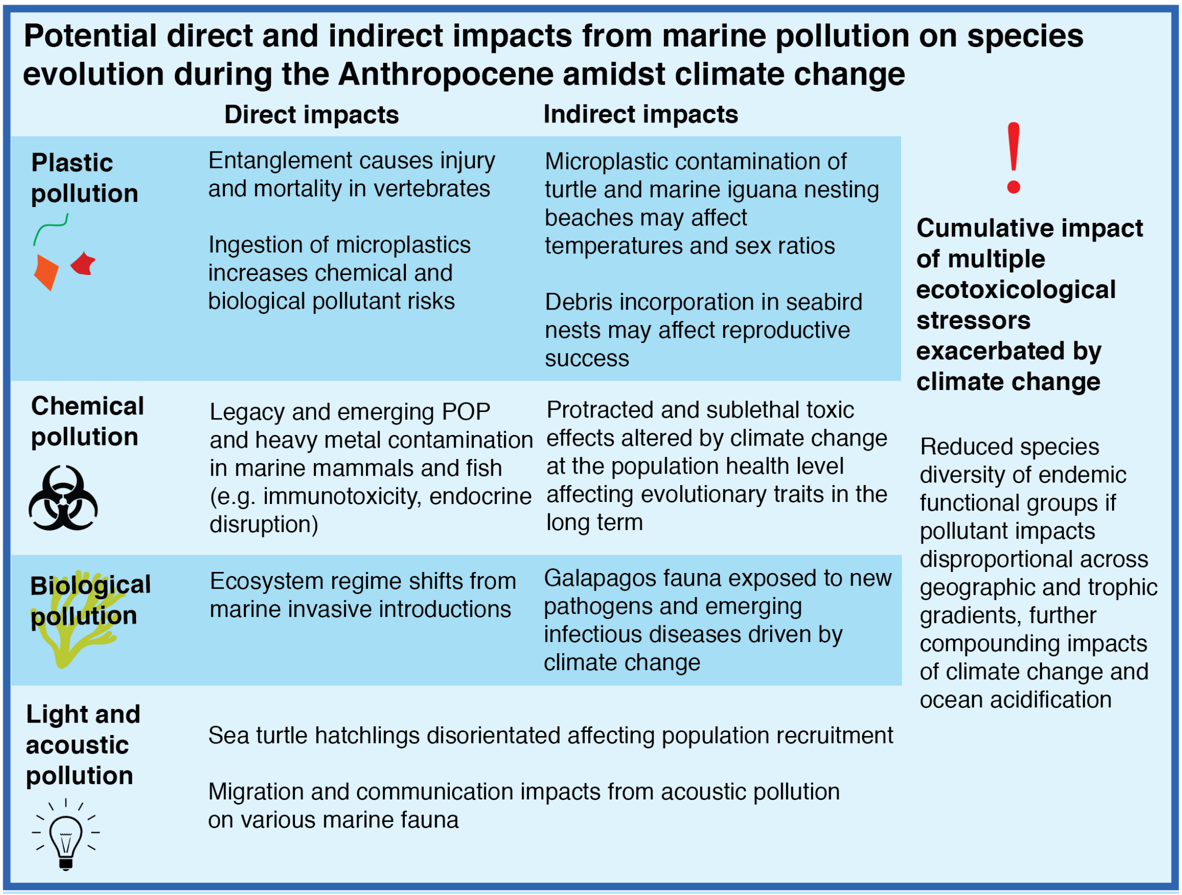

Figure 6.3: Potential direct and indirect impacts from marine pollution on species adaptation amidst climate change. I designed this figure to contribute to a publication in preparation led by Juan Jose Alava: ‘Multiple anthropogenic stressors reshape evolutionary processes in Galápagos: Marine pollution and climate change’.

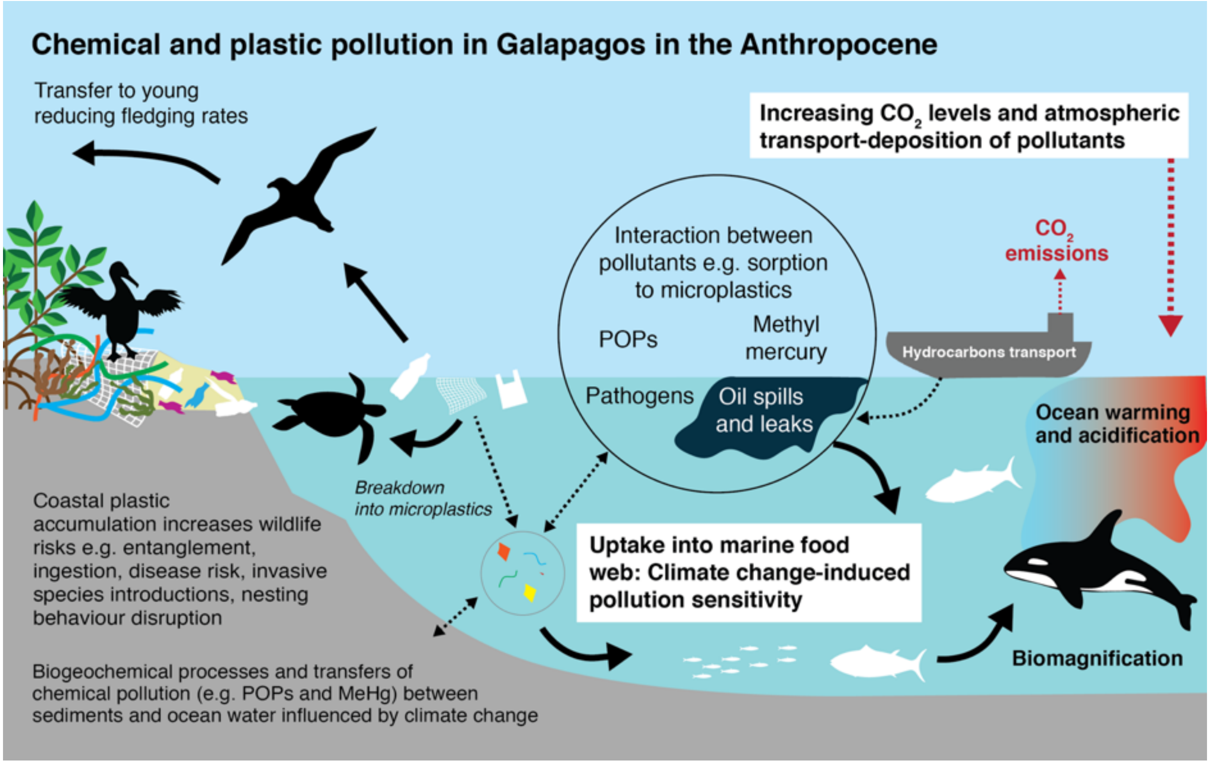

Figure 6.4: Key pathways of marine pollution in Galápagos including oil spills (hydrocarbons), chemical pollution by persistent organic pollutants (POPs) and metals (e.g. methyl mercury, MeHg), biological pollution (e.g. invasive species and pathogens) and marine plastics. I designed this figure to contribute to a publication in preparation led by Juan Jose Alava: 'Multiple anthropogenic stressors reshape evolutionary processes in Galápagos: Marine pollution and climate change'.

Implications for plastic pollution mitigation

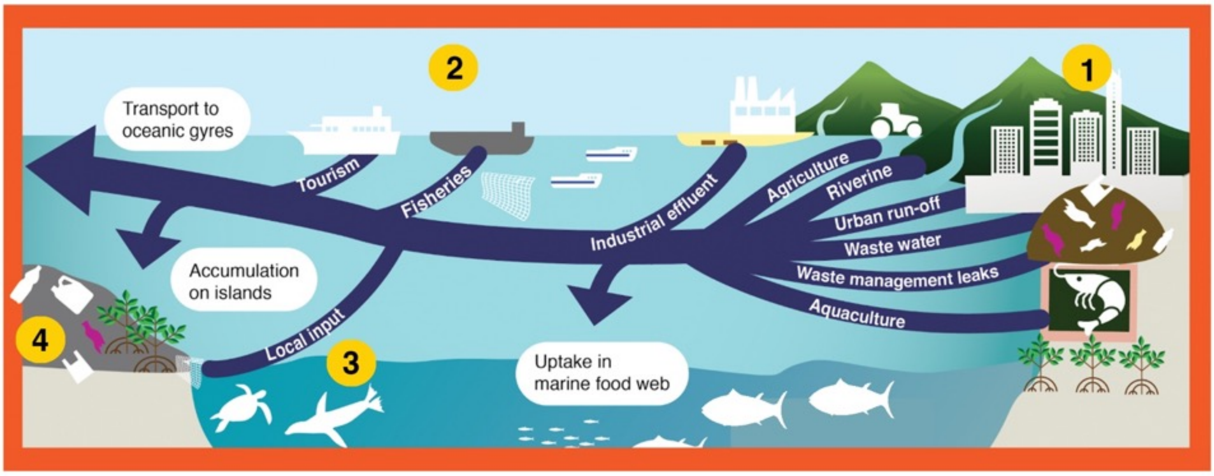

Figure 6.5: Schematic of intervention points to reduce plastic waste on oceanic islands. Upstream interventions supporting a circular economy for plastics and better continental waste management to reduce the inputs (denoted in dark blue arrows) (1), at-sea interventions supporting better waste management primarily in fisheries (2), on-island interventions e.g. improvement in wastewater treatment (3) and remediation through cost-effective, ecologically responsible clean-up (4) ideally adding value to collected plastic waste, linking back with circular economy principles in Intervention 1.

Interventions can be focused on various points along the plastics supply chain. These can be focused on 'turning off the tap' by reducing consumer demand and improving waste management (including minimising littering) to reduce environmental leakage, or can be focused on remediating the issue of plastics already leaked into the environment (i.e. clean up) (Ellen MacArthur Foundation, 2016; Vegter *et al.*, 2014). To reduce plastic waste arriving in Galápagos, my research, along with that of collaborators at the University of Utrecht, University of York and Universidad San Francisco de Quito has highlighted some potential sources where interventions could be targeted (Fig. 6.5). It is likely that a considerable amount of plastics entering the Galápagos Marine Reserve originate from continental sources, suggesting that waste management improvements are needed, particularly along coastal Ecuador and Peru. As in many places around the world, fisheries in the Eastern Pacific appear to be another major source of plastic waste, both via littering of gear but also domestic plastics that are used onboard, as indicated by plastic bottles with Asian lettering being washed up only in the fishing season (as observed by myself during fieldwork and confirmed with local colleagues), a phenomenon also reported in South Atlantic islands (Ryan *et al.*, 2019). The need for waste management improvement in fisheries is imperative to reduce plastic inputs to remote oceanic islands globally (Monteiro *et al.*, 2018; Ryan *et al.*, 2019; Unger & Harrison, 2016), particularly when considering that fishing gear is the most frequently reported source of plastic litter resulting in fatal wildlife entanglements (Jepsen & de Bruyn, 2019; Moore *et al.*, 2009; Parton *et al.*, 2019; Reinert *et al.*, 2017). Finally, improvements in local wastewater treatment may also reduce microplastic contamination that is elevated around populated areas, with interventions such as mechanical filtration technology (Freeman *et al.*, 2020).

Observations suggesting that fragmentation may be happening *in situ* suggest that continued clean-up effort will be important to reduce the future generation of microplastics in addition to reducing the risk of harmful interactions for species. Many accumulation hotspots recognised by my research are not regularly cleaned and are often hard to access, particularly in the rougher waters of the dry/ garua season. The footprint of clean-up is not well documented in Galápagos, both in terms of financial cost (currently the hire of a local fishing boat to access remote sites is > $1,000 (USD) per day) nor in carbon footprint of the fuel required. This is generally funded by NGO grants which is not a sustainable model. The potential of 'at sea' interception of incoming plastic waste has been proposed although it is important to understand potential negative ecological impacts as discussed by Falk-Andersson *et al.* (2020). The authors suggest that fisheries management tools might be applied to measure both the effectiveness of these clean up technologies i.e. catch per unit effort calculations and also to assess ecological effects i.e. employing by-catch reduction strategies (Falk-Andersson *et al.*, 2020). The need to consider post clean up waste management is important, particularly in area with over-burdened systems such as in Galápagos. Even if clean-up becomes more cost-effective through the direction of cleaning effort informed by a combination of our field data confirming hotspots and predictive models to identify optimum timing, there is still the problem of what to do with this waste plastic once collected. It is widely accepted that the adoption of a circular economy for plastics is the key to long-term, systemic change and this approach offers potential channels to add value to cleaned up plastics as well as promoting better future product design (Ellen MacArthur Foundation, 2013, 2016).

Although public and political awareness around plastic pollution has grown considerably over the last few years, this has not yet translated to the systemic changes needed to make a long-term difference in the situation (Pahl, Wyles and Thompson, 2017; Dunn, Mills and Veríssimo, 2020). It is increasingly recognised that individuals, industry and policy-maker actions are not independent but interventions are rarely designed with all stakeholders in mind, embracing behavioural drivers for each group, something necessary to accelerate action beyond solely an economic case (Jia *et al.*, 2019). There has also been recent debate about the relative 'airtime' that plastic pollution has had compared to the more substantial global challenge of climate change (Avery-Gomm *et al.*, 2019; Stafford & Jones, 2019a, 2019b). Something that is not often well communicated is that these two issues are inherently connected, not least due to the vast carbon footprint of plastic production, still primarily manufactured from fossil fuels accounting for 6% of global oil production (Ellen MacArthur Foundation, 2016) with CO_2 emissions along the entire plastics supply chain. By adopting the idea of a 'plastic cycle' as part of the carbon cycle (Zhu, 2021), this may support a more inter-connected view of the drivers and mitigation of both plastic pollution and climate change.

My research supports the general paradigm that plastics can be moved vast distances by oceanic currents and winds, often far from polluting sources, traversing jurisdictions and geographic boundaries requiring regional if not global cooperation across multiple disciplines to tackle the challenge (UNEP, 2016; Vince and Stoett, 2018). Due to the difficulty of tracing sources, there are calls for plastic pollution legislation to be integrated with the treaty on the protection of biodiversity in areas beyond natural jurisdiction (BBNJ) to ensure that action is taken unanimously and at the geographic scale required to minimise risks to biodiversity (Tiller and Nyman, 2018). Technological improvements and better modelling data can support more effective capture and clean-up of leaked plastics but ultimately, the move away from the traditionally linear economy to one that is more circular with products designed with end-of-life in mind will not only contribute to the reduction of plastic pollution, but will also contribute to many of the other United Nations Sustainable Development Goals including the provision of sustainable livelihoods (Ellen MacArthur Foundation, 2016; Lam *et al.*, 2020). To achieve this, we need multi-stakeholder consortia of researchers, industry, government bodies, NGOs and the media to provide a cohesive approach

Next steps with this research

This thesis contains several elements that have and will continue to contribute to conservation and management. Building on results from this body of work, I also present some potential future research questions arising from each chapter to address key knowledge gaps (Table 6.1).

Table 6.1: Potential future research questions

Spatiotemporal distribution of plastic pollution
• Is it possible to differentiate marine-borne plastic pollution from continental and maritime sources? • How much plastic is retained in rocky shore substrates? • How does microplastic move within the three-dimensional beach environment? • What is the role of mangrove systems in plastic pathways and fates in Galápagos?
Biological and ecological impacts
• What is the ecological profile of identified plastic accumulation hotspots? • Do priority scoring analyses incorporating environmental contamination data and observations of wildlife-plastic interactions identify the same species at highest potential risk? • How does the high productivity within the Galápagos Marine Reserve affect plastic particle sinking dynamics? • What are the retention/egestion mechanisms and responses in barnacles and sea cucumbers (as the likely most useful bioindicator species)? • What are the potential impacts of the multi-stressor effects of food limitation and heat stress from ENSO with exposure to microplastic in marine invertebrates? • How does microplastic association vary between different macroalgae species?
Citizen Science
• How useful is citizen science generated data from an applied management perspective? • How effective would the Playas sin Plásticos methodologies be with other citizen scientist groups e.g. tourists? • Does the participation in citizen science data have a positive effect in terms of positive environmental attitudes and more sustainable behavioural choices?

Conclusion

Through this thesis, I have contributed to the understanding of the distribution and composition of plastic contamination in the Galápagos Marine Reserve, confirmed that microplastic is entering the marine food web and highlighted potential species and habitats at higher risk that should be prioritised for future work. Systemic change is required in the long-term with a complete revisioning of economies and supply chains, coupled with innovation to improve waste management and reduce environmental leakage. The regional "Pacific Plastics: Science to Solutions" network emerging in the Eastern Pacific presents a major opportunity in the coming years to pave the way for these changes (Fig. 6.6) but in the meantime, clean-up efforts must continue to reduce the *in situ* fragmentation of items into microplastic that are harder to clean up and are more likely to enter the Galápagos marine food web. Here I have presented data that forms a comprehensive baseline for the current status of microplastic contamination in coastal habitats across the Galápagos Marine Reserve and have developed several tools to support ongoing management of this special ecosystem.

Figure 6.6: "Pacific Plastics: Science to Solutions" Network. Photograph of attendees of the three-day Global Challenges Research Foundation funded workshop in Quito, Ecuador, September 2019 that launched the network with the shared goal of collaborating to reduce plastic waste in the Eastern Pacific.

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Appendix 1: Microplastic photographs from environmental samples

Photographs of representative microplastic particles extracted from beach sand samples (Fig. 7.1), seawater surface samples (Fig. 7.2) and benthic sediment samples (Fig. 7.3). Polymers as identified by FTIR analysis are listed in the figure caption.

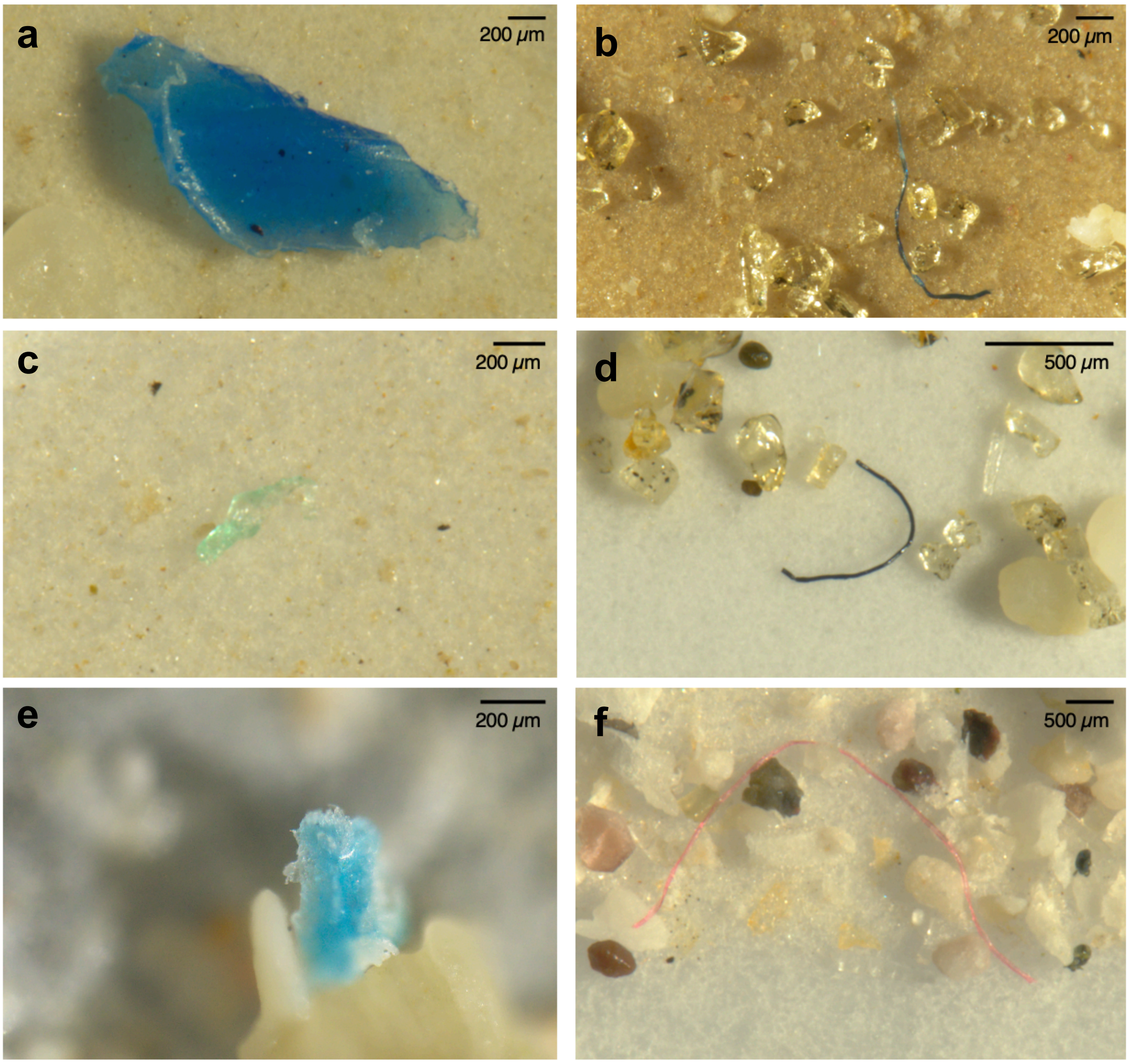

Figure 7.1: Photographs of particles extracted from beach sand samples. a) Blue polyethylene fragment, b) blue cellulosic fibre, c) green polypropylene soft fragment, d) black polyester fibre, e) blue polypropylene fragment, f) red nylon fibre.

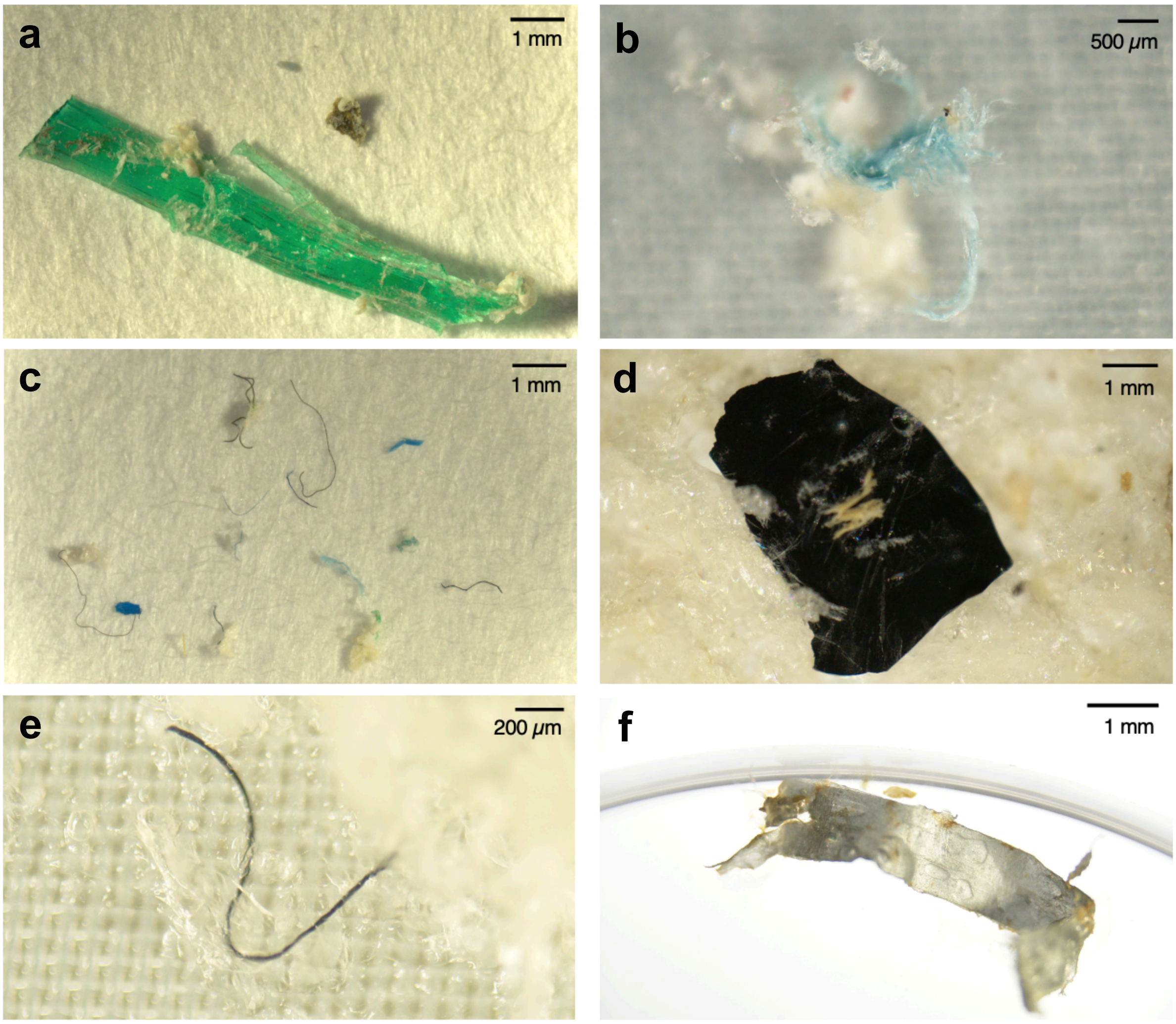

Figure 7.2: Photographs of particles extracted from seawater surface samples. a) Green polypropylene fragment, b) blue polypropylene fragment, c) 12 mixed shape and polymer particles from a water tow sample in San Cristóbal harbour, d) black polyethylene fragment, e) black nylon film, f) silver polyethylene film.

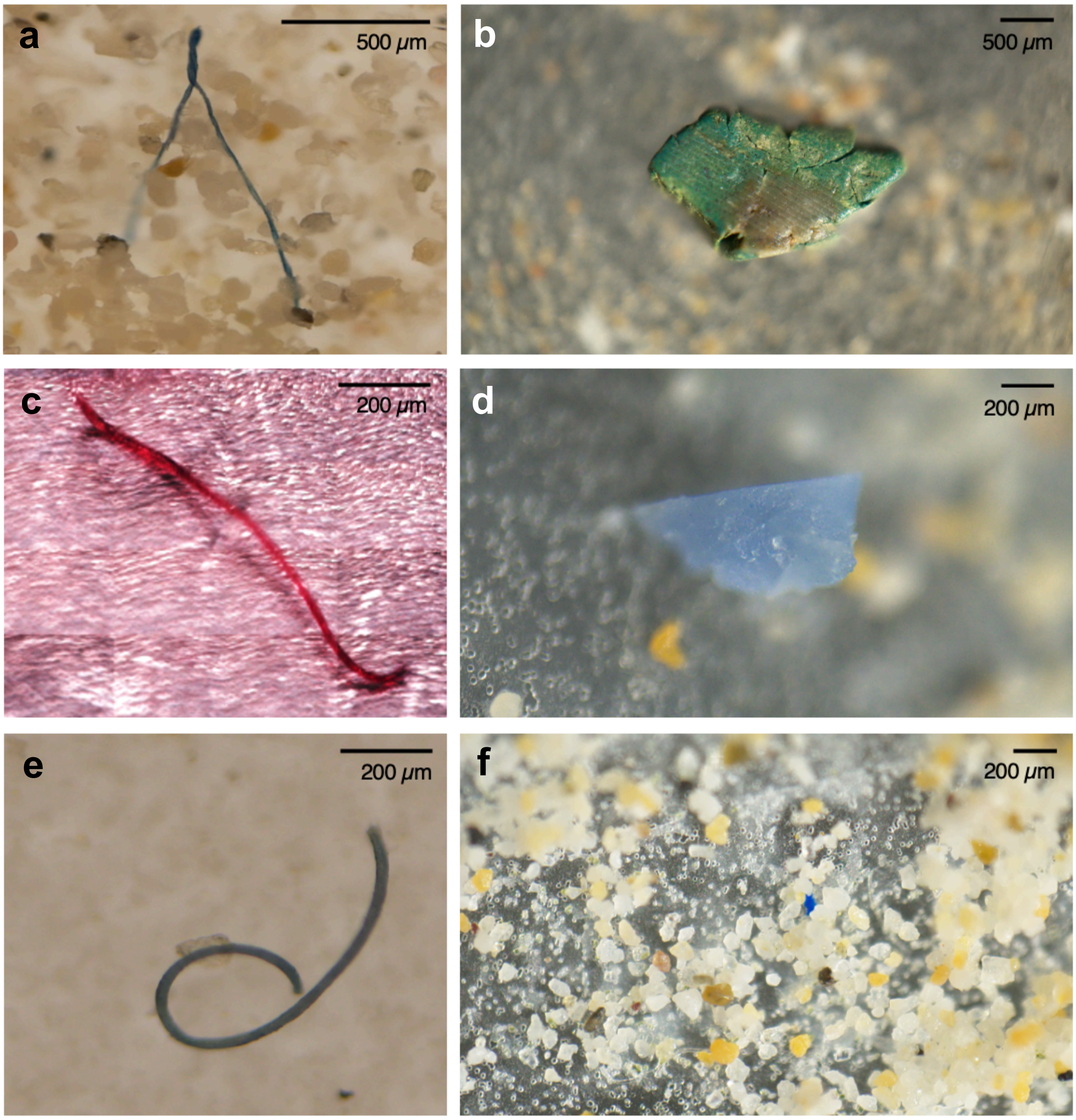

Figure 7.3: Photographs of particles extracted from benthic sediment samples. a) Unidentified blue fibre, b) unidentified green fragment, c) red polyester fibre, d) blue polypropylene fragment, e) black polypropylene fibre, f) unidentified blue fragment, too small to extract.

Appendix 2: Sustainability Statement – A Difficult but Important Conversation…

"In conclusion, it appears that nothing can be more improving to a young naturalist, than a journey in distant countries." **– Charles Darwin**

It is my opinion that as researchers, we are responsible for monitoring the environmental footprint of our work. Although I have not succeeded in doing this comprehensively, i.e. this does not represent a full 'Life Cycle Assessment' of this thesis, I have presented some thoughts here that I wish to include to promote future discussion and transparency.

I have attempted to record all consumable items used during this research to highlight the urgent need for innovation to promote less wasteful practices. Chemicals such as formaldehyde, potassium hydroxide and zinc chloride are all highly toxic but the use of more environmentally friendly alternatives is not always possible and presents problems with inter-study comparisons where standard protocols exist. Some of these chemicals are not permitted to be shipped to the Galápagos Islands, also meaning that finding more sustainable alternatives for microplastic protocols should be a future priority to ensure the future accessibility of this work for local students and researchers, ideally with conversion factors to ensure data are comparable with results from other areas where chemicals are easier to obtain and safely dispose of. In terms of plastic usage, even with being conscious of each item used and re-using items where possible, I estimate that I used a minimum of 3,135 disposable plastic (or synthetic rubber) items during this study, including items such as petri dishes, Nalgene bottles, centrifuge tubes, nylon filter mesh, polycarbonate filters and latex gloves. Following use, these items are destined to be incinerated through standard laboratory disposal systems in the UK and are extremely unlikely to be leaked into the environment. Nonetheless, the production and incineration of plastics has a major climate footprint, in addition to producing toxic by-products that need to be disposed of (Shen *et al*., 2020).

A shift towards disposable plastic consumables has occurred in most laboratories over recent decades, justified due to time and money saved but also in some cases for increased safety and even a reduction of carbon footprint, for example for sample transport where glass would not be a viable alternative. A study from the University of Exeter in 2015 estimated that 280 bench scientists in the Biosciences department used 267 tonnes of plastic in 2014 alone (Urbina *et al*., 2015). In terms of mitigation, they suggested that researchers should pursue ways to re-use plastics in the lab, perhaps possible with the application of stronger contamination controls, and they also recommend that funders should incentivise recycling as part of grant requirements (Urbina *et al*., 2015). The use of autoclavable glassware could perhaps be used more frequently but I observed a substantial increase in contamination when attempting to use glass petri dishes due to the static attraction of microplastic fibres and so discontinued their use. Perhaps innovation of new materials will provide alternatives in the future with a lower carbon footprint e.g. bioplastics. Also, the reduction of background laboratory contamination such as specialised positive pressure cleanrooms dedicated solely to microplastic research, might permit the use of reusable glass and reduce our collective consumables footprint although this of course required substantial investment.

In terms of carbon footprint from travel, I travelled to the Galápagos Islands from the UK three times (May 2018, April 2019, August 2019) to collect the data represented in this thesis, generating estimated emissions of 8.8 tonnes of CO_2 using the calculation method recommended by the Department of Environment for Rural Affairs (DEFRA). This calculation is from the flights alone and does not include the diesel used in boats for 21 days of at sea sampling, the petrol used in car travel nor the footprint of shipping several batches of samples back to the UK. In an effort to maximise the impact of each trip to the Islands, I travelled for 3 – 5 weeks each time to reduce the total number of trips required, undertook work activities including stakeholder meetings and project visits on behalf of my employer, the Galapagos Conservation Trust, and co-delivered capacity strengthening workshops during two of the three trips. I also prioritised in-person public outreach during each trip to support community education programmes and events. On each sampling trip, we invited local researchers and students to provide training to support continued monitoring and future studies, potentially reducing the amount of future travel required from international researchers. In some cases, this supported Ecuadorian students to obtain their undergraduate dissertations or International Baccalaureate (high school) projects, tangibly supporting their scientific education. This theme closely links with the growing awareness of the need to 'decolonise' many areas of science. In agreement with Eichhorn *et al*. (2020), I believe it is our collective responsibility to improve inclusivity and equity within our research fields by increasing access to data, publications, training opportunities and celebrating the reciprocal benefits of collaborations that not only benefit local capacity strengthening but increase the diversity of insights for the interpretation of findings and ultimately the successful design and application of locally relevant interventions (Eichhorn *et al*., 2020).

COVID-19 has forced many of us to reflect on our environmental footprint and rethink what might be possible through a different style of international collaboration. Remote conference attendance via videoconferencing was estimated to reduce climate impact of a case study PhD project by 44% in a study by Achten *et al.* (2013). Although there are clear benefits to connecting with other humans in person, this situation has shown us that it is possible to build very fruitful collaborations and knowledge sharing opportunities remotely (Achten *et al*., 2013). It is important to recognise that some of these digital tools are not available to all potential collaborators globally however and so a mixture of approaches including remote and in-person collaborations are likely required to ensure inclusivity.

It is not possible to establish if this environmental footprint is 'worth it' for the information produced in this thesis, and it is not yet possible to know what the lasting impact of this research might be. Something that is not possible to measure empirically perhaps, is how much I personally and professionally benefitted from this work, meeting new collaborators, experiencing seeing several studies through from design to delivery, giving and receiving training in field techniques, all skills that will be invaluable for planning future scientific conservation programmes. I have been inspired by the commitment of the University of Exeter to improve sustainability practices across the organisation and benefitted from being involved with climate action and lab sustainability groups; connections I intend to continue and lessons I hope to share with our Ecuadorian collaborators. Thanks to this experience, it will now be easier to support other researchers and students remotely. I do feel exceptionally fortunate for this opportunity.

I would like to recommend this exercise of considering the environmental footprint of their projects to current and future PhD students. In addition, I invite funding bodies and universities to consider requesting a sustainability report for thesis projects, particularly when embracing an environmental theme. Perhaps, if this was mapped out at the beginning of a project when considering student training needs and planning fieldwork, we could collectively make a difference, reducing the environmental impact of our work and strengthening capacity and collaborations through our global partnerships.

References for Appendix 2

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Shen, M., Huang, W., Chen, M., Song, B., Zeng, G., & Zhang, Y. (2020). (Micro)plastic crisis: Un-ignorable contribution to global greenhouse gas emissions and climate change. *Journal of Cleaner Production*, *254*, 120138. https://doi.org/10.1016/j.jclepro.2020.120138

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Appendix 3: Communicating and funding this research

Publications/ Manuscripts in Preparation

- **Plastic contamination of a Galapagos Island (Ecuador) and the relative risks to native marine species.** (2021) **Jen S. Jones**, Adam Porter, Juan Pablo Muñoz-Pérez, Daniela Alarcón-Ruales, Tamara S. Galloway, Brendan J. Godley, David Santillo, Jessica Vagg & Ceri Lewis. *Science of the Total Environment, 789. https://doi.org/10.1016/j.scitotenv.2021.147704.* (**Chapter 2**).
- **Microplastic pollution on two Galápagos Island beaches, Ecuador: An assessment using citizen science derived data. Jen S. Jones**, Anne Guézou, Sara Medor, Caitlin Nickson, Georgian Savage, Daniela Alarcón-Ruales, Tamara S. Galloway, Juan Pablo Muñoz-Pérez, Sarah E. Nelms, Adam Porter, Martin Thiel & Ceri Lewis. *Manuscript in preparation.*
- **Contemporary Archaeology as a framework for investigating the impact of disposable plastic bags on environmental pollution in Galápagos.** (2021) John Schofield, Jerry Aylmer, Andy Donnelly, **Jen Jones**, Juan Pablo Muñoz-Pérez, Elena Perez, Callum Scott & Kathy A. Townsend. *Journal of Contemporary Archaeology* Vol. 7, pp. 276 – 306. *In press.*
- **Object narratives as a methodology for mitigating marine plastic pollution: multidisciplinary investigations in Galápagos.** (2020) John Schofield, Kayleigh J. Wyles, Sean Doherty, Andy Donnelly, **Jen Jones** & Adam Porter. *Antiquity* Vol. 94, pp. 228 – 244. https://doi.org/10.15184/aqy.2019.232
- **Basin-scale sources and pathways of microplastic that ends up in the Galápagos Archipelago**. Erik van Sebille, Philippe Delandmeter, John Schofield, Britta Denise Hardesty, **Jen Jones** & Andy Donnelly (2019) *Ocean Science* Vol. 15, pp. 1341 – 1349. https://doi:org/10.5194/os-2019-37

Awards and grants

- **Reducing the impacts of plastic waste in the Eastern Pacific** (2020) Principal Investigators: Professor Tamara Galloway & Dr Ceri Lewis, University of Exeter, **£3,562,137** (Global Challenges Research Fund, **NE**/V005448/1)
    - **Role:** I led the design of the programme i.e. Theory of Change, liaised with partners to agree objectives and deliverables, supported the formation of research questions and co-managed the proposal writing process.
- **Science to Solutions: Collaborating to reduce plastic pollution in the Eastern Pacific** (2019) Principal Investigators: Professor Tamara Galloway & Dr Carlos Mena, **£25,000** (Global Challenges Research Fund Networking Grant)
    - **Role:** I co-designed the networking activity plans including approaching partners, managing workshop administration and facilitation, arranged and co-supervised students on knowledge exchange visits to the University of Exeter and supported proposal writing.
- **Collaborating for a Marine Litter Management Plan for Galápagos** (2018) Project Leader: **Jen Jones** £30,670 (The Woodspring Trust)
- **Conservation of the endemic Galápagos marine iguana: quantifying the emerging threat of marine plastic pollution** (2019) Project Leaders: **Jen Jones** & Juan Pablo Muñoz-Pérez $11,350 (USD) (International Iguana Foundation)
- **Playas Sin Plásticos** (2019) Project Leaders: **Jen Jones** & Daniela Alarcón £2,500 *in kind boat time* (Galápagos Sharksky Travel & Conservation)
- **Playas Sin Plásticos** (2019) Project Leaders: **Jen Jones** & Daniela Alarcón £1,000 (British Embassy of Quito, Ecuador)

Conferences

- **Towards more plasticity in plastics research: a case study from the Galápagos Islands, Ecuador. Jen S. Jones** (presenter), Diana A. Pazmiño, Elena M. Pérez.

Celebrating Diversity in Science Conference (*best PhD speaker award*), December 2020.

- **Investigating the risks of plastic pollution in the Galápagos Marine Reserve, Ecuador**. **Jen S. Jones** (presenter), Adam Porter, Juan Pablo Muñoz-Pérez, Daniela Alarcón-Ruales, Tamara S. Galloway, Brendan J. Godley, David Santillo, Jessica Vagg, Ceri Lewis. MICRO2020, November 2020.
- **From Science to Solutions: Investigating the Impacts of Marine Litter on the Galápagos Marine Food web. Jen S. Jones** (presenter), MICRO2018, November 2018.
- **Defining the Risk of Microplastics to Species in the Galápagos Marine Reserve. Jen S. Jones** (presenter), CLESCon (University of Exeter), October 2018.

Scientific meetings

Co-designer and facilitator:

- **Science to Solutions: Collaborating to tackle plastic pollution in the Eastern Pacific**. Universidad San Francisco de Quito, Ecuador, September 2019
- **Science to Solutions: Towards a Plastic Pollution Free Galápagos.** Galápagos Science Center/ Charles Darwin Foundation, Galápagos Islands, May 2018

Public Outreach

- 'Plastic Pollution Free Galápagos Webinar' – speaker with Erik Van Sebille for an online event ran by the Galápagos Conservation Trust, September 2020
- 'Highland Ocean Plastics Webinar 2020' – speaker for an international event ran by Green Hive Scotland, August 2020

- ‘Investigating microplastics in the Galápagos Marine Reserve’ – public talk for the San Cristóbal island community at the Galápagos Science Center, April 2019
- Local radio interview on ‘Radio Encantada’ with colleagues from the Galápagos Science Center – April 2019
- ‘The plastic hunters’ – talk for Exeter Café Scientifique - October 2018
- ‘Even the Galápagos Islands are being polluted by plastic’ - ITV news, May 2018

"You cannot get through a single day without having an impact on the world around you.

What you do makes a difference, and you have to decide what kind of difference you want to make."

Dr Jane Goodall, DBE

Supporting Science for a
#PlasticPollutionFreeGalapagos