# Marine litter, microplastics and marine megafauna

Submitted by

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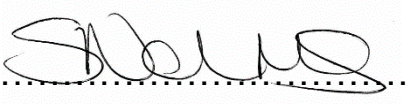
Signature:  ……..

Sarah E. Nelms

Grey seal (*Halichoerus grypus*) pup surrounded by plastic pollution in the Firth of Forth, Scotland (Photo: Matt Carter).

## Abstract

Over the last sixty years, the development of synthetic and durable materials, namely plastic, coupled with a growing human population, has resulted in a rapid increase in the levels of anthropogenic debris in rivers, along coastlines and in the wider marine ecosystem. Currently, an estimated 4.8 to 12.7 million tons of plastic enter the oceans every year but this is expected to increase to between 9.6 and 25.4 million tons by 2025. As such, it is one of the most widely recognised pollution issues facing the planet due to its wide-ranging ecological and socio-economic implications. The main aims of this thesis were to i) examine citizen-science beach clean data to better understand the composition of anthropogenic litter deposited on British beaches by determining the most common items, materials, sources and pathways, and exploring the data for spatial patterns and temporal trends in litter density; ii) investigate an indirect pathway (trophic transfer) of microplastic (<5mm in size) ingestion in marine top predators by analysing scat (faeces) from captive grey seals (*Halichoerus grypus*) and the wild-caught fish they were fed upon; iii) explore the extent to which wild marine mammals ingest microplastics and consider the potential implications by examining the digestive tracts of 50 marine mammals from 10 species that stranded around the British coast; iv) develop a method of investigating dietary exposure of marine mammal top predators to microplastics, by combining scat-based molecular techniques (metabarcoding) with a microplastic isolation method. The research carried out for this thesis reveals that i) plastic is the main constituent of marine litter on British beaches and the majority of traceable items originate from land-based activities, such as public littering. The coasts of the southwest England and south Wales have the highest litter levels and certain items - small plastic fragments, plastic food packaging, wet wipes, polystyrene foam, balloons and large fishing net – are increasing; ii) trophic transfer is an indirect and under-studied, but potentially major, route of microplastic ingestion for marine top predators; iii) microplastics are ubiquitous within the digestive tracts of wild marine mammals stranded around the British coast but the overall low abundance suggests they may be egested; iv) the rate of microplastic ingestion by marine top predators may be related to the type of prey they consume but further work is needed to assess the impacts of this omnipresent pollutant.

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To my supervisors, I wish to express my most heartfelt gratitude. The trust and faith of Dr Penelope (Pennie) Lindeque when taking me on as an additional (and sometimes rogue) PhD student humbles me greatly. Without her, this PhD would not have come about and I am immensely grateful to her for taking a gamble on me. Her relaxed and fun nature has taught me that, sometimes, the best science ideas come from chats in the sun, looking out to sea. The support and guidance of Professor Brendan Godley has been instrumental in this journey into academia and his mentorship, from the day I became his MSc student five and a half years ago, has been the driving force in building my confidence and independence as a scientist. I have been so incredibly lucky to receive his encouragement and reassurances when things got tough, and patience when it was needed. My interest in microplastics began when Professor Tamara Galloway delivered a seminar during my masters. Her research opened my eyes to the issue and inspired me to want to know more. I am grateful to her for encouraging my curiosity and offering positive advice when needed. I thank you all for enabling me to combine my interests and your expertise into a PhD which has been a challenging, but immensely rewarding, experience. You have given me the space to determine the direction of my own research and grow as a result. I sincerely hope that the last three and half years have been as enjoyable for you as they have been for me.

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I am indebted to you all.

*I need the sea because it teaches me.*
*I don't know if I learn music or awareness,*
*If it's a single wave or its vast existence,*
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- The Sea, Pablo Neruda

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## Author's declaration

My thesis is presented as four research papers (three published and one submitted). All work has been the product of my planning and implementation, and I am the lead author on all the papers presented here; the contributions from my supervisors and co-authors are described at the beginning of each chapter. Papers have been reformatted to provide a unified editorial and referencing style throughout, with figures embedded within the text. References are compiled into a single bibliography at the end of the thesis.

## List of definitions and abbreviations

Cetaceans: Dolphins and whales

FTIR: Fourier-Transform Infrared spectroscopy

GIT: Gastro-intestinal tract (gut)

HTS: High-throughput sequencing

Macroplastic: Plastic larger than 5 mm in size

Microplastic: Plastic smaller than 5 mm in size

µm: Micrometre

mL: Millilitre

mm: Millimetre

Nanoplastic: Plastic smaller than 1 µm in size

OCPs: Organochlorine pesticides

PCBs: Polychlorinated biphenyls

PCR: Polymerase Chain Reaction

Pinnipeds: Seals, fur seals, sea lions, walruses

POPs: Persistent organic pollutants

Scat: Faeces

# General Introduction

*Marine anthropogenic litter*

Increasing human reliance on global marine environments for resources and space is exerting significant and expanding detrimental impacts upon species and habitats (Crain et al., 2008; Halpern et al., 2015). Anthropogenic stressors such as climate change, over-exploitation and pollution have led to widespread habitat degradation and biodiversity loss (Halpern et al., 2015; Parsons et al., 2014). Marine anthropogenic litter - defined as 'any persistent, manufactured or processed material discarded, disposed of or abandoned in the marine and coastal environment' – has become one of the most well-known pollution issues facing the world's waterways and oceans (Lippiatt et al., 2013). It is a complex, trans-boundary and cross-sectoral issue with wide-ranging economic, social and ecological implications (Hastings and Potts, 2013; Kuhn et al., 2015; Newman et al., 2015; UNEP, 2016; Wyles et al., 2015). Over the last sixty years, the development of synthetic and durable materials, namely plastic, coupled with a growing human population, has resulted in a rapid increase in the levels of debris in rivers, along coastlines and in the wider marine ecosystem.

Between 4.8 and 12.7 million metric tons of plastic are estimated to enter the oceans every year (Jambeck et al., 2015), via a variety of pathways, including public littering, fly-tipping, sewage and poor waste management (Duckett et al., 2015; Galgani et al., 2013; Poeta et al., 2014), as well as direct input from maritime industries, such as fishing and shipping (Galgani et al., 2013; Hastings and Potts, 2013; Moriarty et al., 2016; Nelms et al., 2017). Since plastic does not biodegrade and persists for an unknown amount of time, the amount accumulating in marine ecosystems is increasing exponentially (Barnes et al., 2009). Plastic does, however, degrade and fragment into microplastics (<5 mm in size), as a result of solar ultraviolet (UV) radiation, wave action and physical abrasion (Andrady, 2011; Barnes et al., 2009). Additional sources of microplastics include pre-production pellets (nurdles) spilled during transportation and fabrication, outflow of wastewater containing microbeads from cosmetics and fibres from the washing of synthetic textiles, as well as road-run-off containing fragments of vehicle tyres and road marking paint (Andrady, 2011; Barnes et al., 2009; Boucher and Friot, 2017; Browne et al., 2011; Napper et al., 2015; Napper and Thompson, 2016; UNEP, 2009).

*Plastic pollution and marine megafauna*

Due to its omnipresence within the world's ocean, interactions between plastic and marine megafauna are prevalent. For example, all (100%) of the seven sea turtle species, 53% of the 346 seabird species and 41% of the 128 marine mammal species are known to be affected (Nelms et al., 2016; Senko et al., In review; see Appendix 2). The three main ecological impacts are entanglement, ingestion and habitat degradation. Entanglement in anthropogenic items, such as derelict fishing gear, sheet plastic and strapping can cause amputation of limbs, strangulation, increased drag and the associated energetic costs, a reduced ability to avoid predators or forage, starvation and drowning (Duncan et al., 2017; Votier et al., 2011; Senko et al., In review). Ingestion of macroplastics (> 5 mm) may lead to blockages and impaction of the digestive tract, laceration of the stomach and intestinal walls by hard/ sharp plastic, dietary dilution due to a false sense of satiation, dehydration and starvation (Nelms et al., 2016). The extent of damage caused by plastic pollution to sensitive habitats, such as coral reefs, mangroves and sea grass beds, is not well understood but impacts include smothering, sedimentation, coral breakages and increased vulnerability to disease (Gregory, 2009; Lamb et al., 2018).

*Microplastic ingestion in marine mammals*

Due to their small size, microplastics are highly bioavailable to a wide variety of marine biota from zooplankton, such as copepods, other invertebrates (including shellfish), both juvenile and adult fish, seabirds and marine megafauna (Amélineau et al., 2016; Besseling et al., 2015; Cole et al., 2013; Desforges et al., 2015; Farrell and Nelson, 2013; Lusher et al., 2015, 2013; Steer et al., 2017; Watts et al., 2014). Microplastic ingestion in low trophic level organisms, such as zooplankton, molluscs, polychaete worms and fish, can cause a reduction in feeding capacity, energy reserves and reproductive output as well as detrimental alterations to intestinal function (Cole et al., 2015; Pedà et al., 2016; Sussarellu et al., 2016; Wright et al., 2013a). Yet, there is a need to better understand the extent to which microplastics are ingested by high trophic-level taxa, for example marine mammals, and whether any health implications occur as a result.

Marine mammals, such as whales, dolphins and seals, are often considered sentinels for marine ecosystem health, particularly in relation to pollution (Bossart, 2011; Mössner and Ballschmiter, 1997). The high-trophic level status

and long life-span of some species leave them susceptible to bioaccumulation and biomagnification of aquatic chemical contaminants, which have been shown to cause population-level effects (Jepson et al., 2016; Murphy et al., 2015; Pierce et al., 2008). As a result of this, and other anthropogenic stressors, many species of this taxonomic group are of conservation concern (Parsons et al., 2015). Ingestion of anthropogenic litter by marine mammals has been documented in a variety of species (Kuhn et al., 2015), yet the number of studies (which use appropriate methods of extraction and contamination control) investigating the physical presence of microplastics in the digestive tracts of wild cetaceans and pinnipeds, although growing, is still low (Besseling et al., 2015; Hernandez-Gonzalez et al., 2018; Lusher et al., 2015, 2018; Nelms et al., 2019; Xiong et al., 2018). Microplastics may be ingested directly due to accidental consumption, for example as a result of indiscriminate feeding strategies, such as filter-feeding (e.g. mysticete whales; Besseling et al., 2015) or indirectly as a result of trophic transfer, whereby predators consume prey items containing microplastics (Farrell and Nelson, 2013), for example, during raptorial feeding (e.g. most seals and dolphins; Hocking et al., 2017). Little is known about the extent of microplastic ingestion, and the mechanisms that cause it, in marine mammals.

In this thesis, '***Marine litter, microplastics and marine megafauna'***, throughout four chapters written as independent units, I explore the suitability of citizen-science data for assessing the distribution and abundance of anthropogenic litter in coastal environments, and investigate the mechanisms and extent of plastic ingestion, specifically microplastics, in marine mammal top predators.

In Chapter 1, '***Marine anthropogenic litter on British beaches: A 10-year nationwide assessment using citizen science data'***, I examine the composition of litter deposited on beaches by item type, material, source and pathway, and explore the data for spatial patterns and temporal trends in litter density. The results reveal that plastic is the main constituent of marine litter on British beaches and the majority of traceable items originate from land-based sources, such as public littering. The coast of the southwest England and south Wales is identified as having the highest litter levels. Increasing trends over the 10-year time period were detected for a number of individual item categories, yet no statistically significant change in overall litter was detected. The limitations of the dataset are discussed and I make recommendations for future work.

In Chapter 2, '***Investigating microplastic trophic transfer in a marine top predator****',* I overcome the logistical and ethical constraints of investigating the transfer of microplastics from prey to marine top predator by analysing scat (faeces) from captive grey seals (*Halichoerus grypus*) and the wild-caught fish they are fed upon. I found that approximately half of the scats and a third of fish contained between one and four microplastics and ethylene propylene was the most frequently detected polymer type in both. This result suggests that trophic transfer represents an indirect, yet potentially major, pathway of microplastic ingestion for marine top predators. This microplastic ingestion pathway was further investigated in Chapter 4, '***What goes in, must come out: combining scat-based molecular diet analysis and quantification of ingested microplastics in a marine top predator, the grey seal (Halichoerus grypus)***'. Here I develop a novel and effective methodology pipeline to investigate dietary exposure of wild top predators (grey seals) to microplastics. To do so, I employ DNA metabarcoding, a rapid method of biodiversity assessment, to garner detailed information on prey composition from scats, and investigated the potential relationship between diet and microplastic burden. Outcomes of the method development process and results of both diet composition from metabarcoding analysis and detection of microplastics are presented. Importantly, the pipeline performed well and initial results suggest the frequency of microplastics detected in seal scats may be related to the type of prey consumed.

In Chapter 3, '***Microplastics in marine mammals stranded around the British coast: ubiquitous but transitory?***', I perform a comprehensive assessment of microplastic ingestion in wild marine mammals by examining the whole digestive tracts of 50 individuals from 10 species that stranded around the coastline of Britain. I found at least one microplastic in every animal I examined. The relatively low number per animal (mean = 5.5), however, suggests these particles are transitory. Even so, stomachs were found to contain a greater number than intestines, indicating a potential site of temporary retention. A possible relationship was found between the cause of death category and microplastic abundance, indicating that animals that died due to infectious diseases had a slightly higher number of particles than those that died of trauma and other drivers of mortality. I discuss possible reasons for, and implications of, microplastic ingestion by these animals.

In addition to the chapters listed above, I have contributed to two reviews outlining the impacts of plastic pollution on marine megafauna as an aside to this thesis – ‘Plastic and marine turtles: a review and call for research’ (lead author; published) and ‘Global impacts of plastic pollution on air-breathing marine megafauna: A review with emerging research priorities’ (second author; in review), which I include as appendices (Appendix 1 and 2 respectively).

# Chapter 1: Marine anthropogenic litter on British beaches: a 10-year nationwide assessment using citizen science data

This chapter is a reformatted copy of my publication: **Nelms SE**, Coombes C, Foster LC, Galloway TS, Godley BJ, Lindeque PK, Witt MJ (2017). Marine litter on British beaches: a 10-year nationwide assessment using citizen science data. *Science of the Total Environment*. 579: 1399-1409. I conducted all of the analysis and was lead author on this work; MW guided the development of the analysis and writing; CC and LCF provided the data; all authors provided comments and edits to help shape the final manuscript. **Impact: Citations – 54; Altmetric score - 386**


## Abstract

Growing evidence suggests that anthropogenic litter, particularly plastic, represents a highly pervasive and persistent threat to global marine ecosystems. Multinational research is progressing to characterise its sources, distribution and abundance so that interventions aimed at reducing future inputs and clearing extant litter can be developed. Citizen science projects, whereby members of the public gather information, offer a low-cost method of collecting large volumes of data with considerable temporal and spatial coverage. Furthermore, such projects raise awareness of environmental issues and can lead to positive changes in behaviours and attitudes. We present data collected over a decade (2005-2014 inclusive) by Marine Conservation Society (MCS) volunteers during beach litter surveys carried out along the British coastline, with the aim of increasing knowledge on the composition, spatial distribution and temporal trends of coastal debris. Unlike many citizen science projects, the MCS beach litter survey programme gathers information on the number of volunteers, duration of surveys and distances covered. This comprehensive information provides an opportunity to standardise data for variation in sampling effort among surveys, enhancing the value of outputs and robustness of findings. We found that plastic is the main constituent of anthropogenic litter on British beaches and the majority of traceable items originate from land-based sources, such as public littering. We identify the coast of the Western English Channel and Celtic Sea as experiencing the highest relative litter levels. Increasing trends over the 10-year time period were detected for a number of individual item categories, yet no statistically significant change in total (effort-corrected) litter was detected. We discuss the

limitations of the dataset and make recommendations for future work. The study demonstrates the value of citizen science data in providing insights that would otherwise not be possible due to logistical and financial constraints of running government-funded sampling programmes on such large scales.

## Introduction

Pollution of the marine environment by anthropogenic litter is now widely acknowledged as a significant global environmental issue requiring mitigation (Cole et al., 2011; Derraik, 2002; Vegter et al., 2014). Defined as 'any persistent, manufactured or processed material discarded, disposed of or abandoned in the marine and coastal environment', anthropogenic litter is a complex, trans-boundary and cross-sectoral concern (Hastings and Potts, 2013; UNEP, 2009). Originating from both marine- and land-based activities, the sources of debris are numerous and extensive (UNEP, 2016). Inputs from maritime activities, such as commercial and recreational fisheries and shipping, include items such as ropes, cages, nets, fishing line, plastic fish boxes, floats and buoys (Galgani et al., 2013; Moriarty et al., 2016). Items from land-based sources originate from domestic, industrial and agricultural activities (UNEP, 2009) and may enter the marine environment via a variety of pathways, including public littering, fly-tipping and poor waste management (Hastings and Potts, 2013; UNEP, 2009), transported to the sea by rivers, sewage outflows and wind (Duckett et al., 2015; Galgani et al., 2013; Poeta et al., 2014; Rech et al., 2014). Anthropogenic factors, such as proximity to areas of high population density, degree of fishing effort and concentration of shipping traffic, are likely to affect the abundance and distribution of debris (Duckett et al., 2015; Hoellein et al., 2015; Moriarty et al., 2016; Ribic et al., 2012). Furthermore, environmental factors, such as wind, tides, currents and coastal morphology, are influential in the distribution and accumulation of marine anthropogenic litter (Critchell et al., 2015), but are complex and their precise effects are difficult to disentangle (Browne et al., 2015).

In most cases, plastic is the main constituent of marine anthropogenic litter (Barnes et al., 2009; Derraik, 2002; Poeta et al., 2014; Schulz et al., 2015; UNEP, 2009). This is due partly to its expanding popularity as a consumer product, and its high durability and persistence within the marine environment (Andrady, 2015; Barnes et al., 2009; Jambeck et al., 2015). This synthetic material does not biodegrade but only fragments into smaller pieces (Sigler, 2014). Whilst near the

sea-surface or on a beach, plastic is photo-degraded by solar ultraviolet (UV) radiation (Andrady, 2015). Once weakened, larger macro-plastics are fragmented by wave action and physical abrasion, eventually becoming micro-plastics (typically defined as items <5 mm in size; Andrady, 2011; Barnes et al., 2009). Additionally, some plastics that are produced specifically to be of a small size, such as pre-production pellets (nurdles*)* and polystyrene beads, microbeads from cosmetics and microfibers released during the washing of textiles, enter the marine environment directly through spills or sewerage systems (Browne et al., 2011; Cole et al., 2011; UNEP, 2009). Due to their low-density, many types of plastic are buoyant, which enables transport around global oceans via wind and current driven surface circulation, dispersing them over large distances far from their site of origin. This makes it challenging to identify their sources and implement focused management activities (Barnes et al., 2009).

Persistent marine debris, including plastics, has a range of environmental, economic and social impacts (UNEP, 2016). For biodiversity, detrimental effects include ingestion of both macro- and micro-debris (Cole et al., 2013; Lusher et al., 2015; Nelms et al., 2016; Vegter et al., 2014); entanglement in netting, sheet plastic and packing materials (Bentivegna, 1995; Chatto, 1995; Votier et al., 2011); habitat degradation and alteration by smothering (Carson et al., 2011; Richards and Beger, 2011) and transport of invasive species (Kiessling et al., 2015). Furthermore, plastics are susceptible to the adsorption of hydrophobic contaminants (Teuten et al., 2007), such as heavy metals and polychlorinated biphenyls (PCBs), from the surrounding seawater (Endo et al., 2005; Rochman et al., 2014). If ingested, these toxic compounds, and others incorporated during production (such as plasticizers), may be released into biological tissue, potentially causing cryptic, sub-lethal effects for the organism (Batel et al., 2016; Laing et al., 2016).

Marine and coastal ecosystems are important economically, through industries such as fisheries and tourism, and socially, i.e. benefits to health and well-being (Martínez et al., 2007; White et al., 2014). The presence of anthropogenic litter, however, can diminish these returns. For example, in the United Kingdom (UK), the economic cost to fisheries is estimated at £10 million per year (e.g. repair of gear damaged by debris, time lost due to removal and repairs) and local authorities spend approximately £15 million annually on the removal of beach litter (Hastings and Potts, 2013; Mouat et al., 2010; Newman et al., 2015). The

aesthetic impact of anthropogenic litter has implications for tourism and human well-being. For example, 85% of 1000 residents and tourists said they would not visit a beach with an excess of two litter items per metre (Ballance et al., 2000; Hastings and Potts, 2013), and Tudor and Williams (2006) reported that beach choice was more strongly determined by clean, litter-free sand and seawater than by safety. Wyles et al. (2015) found that the restorative psychological benefits ordinarily experienced by people visiting the coast were undermined by the presence of litter.

To understand the scale of the marine anthropogenic litter problem and inform the development of effective management strategies, it is necessary to conduct monitoring programmes that follow trends in levels of pollution as well as identify pathways and sources (Critchell and Lambrechts, 2016; Rosevelt et al., 2013; Schulz et al., 2015). In the European Union (EU), such monitoring is required of member states by the Marine Strategy Framework Directive which aims to achieve Good Environmental Status (GES) of EU marine waters by 2020 (Moriarty et al., 2016; MSFD GES Technical Subgroup on Marine Litter, 2011). Beach litter surveys are a well-known technique for gathering information on the status of anthropogenic litter, both for the beaches themselves, and as an indicator for the wider marine environment (Ribic et al., 2012). OSPAR (The Convention for the Protection of the Marine Environment of the North-East Atlantic) has been monitoring 50 indicator beaches (located within six OSPAR regions in the North-East Atlantic) using a standardised protocol since 1998. These beaches are surveyed four times a year (at three month intervals) and the number of litter items per 100 m of coastline recorded (OSPAR, 2010). Such endeavours, however, require considerable time and resources to collect meaningful and robust data. Volunteers are often recruited to carry out beach litter surveys and their involvement as *citizen scientists* can be instrumental in the generation of large, long-term datasets which may otherwise not be feasible due to logistical or financial constraints (Duckett et al., 2015; Hidalgo-Ruz and Thiel, 2015, 2013). The inclusion of people of all ages from a broad social spectrum reduces the time and cost of sampling, raises awareness of environmental issues within the wider community and may lead to positive changes in behaviours and attitudes (Wyles et al., 2016). The information generated can be used to develop practical solutions at local, regional and potentially even global scales (Browne et al., 2015; Munari et al., 2015; Ribic et al., 2012; Rosevelt et al., 2013; van der

Velde et al., 2016). The results of very few (non-research focussed) beach litter programmes reach peer-reviewed scientific journals (Browne et al., 2015). This may be due to logistical or administrative constraints but is also likely related to limitations in some citizen science projects, such as lack of information on survey effort, the absence of standard methods to ensure comparability among surveys and lack of links between non-governmental organisations (NGOs) and academic institutions (Duckett et al., 2015; Hidalgo-Ruz and Thiel, 2015). With appropriately designed sampling protocols (for example, prior standardisation of survey effort) and rigorous analyses it becomes possible to ameliorate some of these concerns (Duckett et al., 2015; Hidalgo-Ruz and Thiel, 2015; van der Velde et al., 2016).

Each year, the Marine Conservation Society (MCS) – a UK-based charity focused on improving marine stewardship and public engagement – runs a national volunteer beach litter surveying programme around the British coastline. In this study we analysed 10 years of beach litter data collected during the period 2005-2014 (inclusive). The aims of this study were to: 1) determine composition of litter (by item category, material, pathway and origin); 2) investigate spatial patterns (on a regional scale) 3) explore temporal trends in abundance of overall litter and individual item categories and 4) based on findings, produce recommendations for future work with the aim of enhancing the field of marine litter research and public engagement.

## Materials and methods

*Study region*

Along the eastern and southern borders of Britain are the North Sea and the English Channel. The former is a semi-enclosed shelf-sea, surrounded by seven countries (Britain, France, Belgium, Netherlands, Germany, Denmark and Norway) and connected to the Atlantic Ocean through the English Channel to the south and the Norwegian sea to the north (Huthnance, 1991). Along the western border are the Celtic Seas, which fringe the western coastlines of Scotland and England and the entirety of Wales. This region contains oceanic water from the North Atlantic which enters from the south and west and predominantly moves northwards (http://www.ospar.org/convention/the-north-east-atlantic/iii; last accessed 8 August 2016). The prevailing wind direction is from the south-west, with considerable seasonal and regional variability in speed and direction.

*Beach litter surveys*

Data on marine anthropogenic litter were collected by MCS volunteers between January 2005 and December 2014 (inclusive) from 736 beaches located throughout Britain, in England, Scotland and Wales (see Fig. 1). For the purposes of regional analysis, beaches were assigned to one of seven Regional Seas areas, as designated by the Joint Nature Conservation Committee (JNCC; UK) based on biogeographical characteristics (http://jncc.defra.gov.uk/page-1612; last accessed 8 August 2016). These are; Northern North Sea (NNS), Southern North Sea (SNS), Eastern English Channel (EEC), Western English Channel and Celtic Sea (WECCS), Irish Sea (IS), Minches and West Scotland (MWS), Scottish Continental Shelf (SCS; Fig. 1).

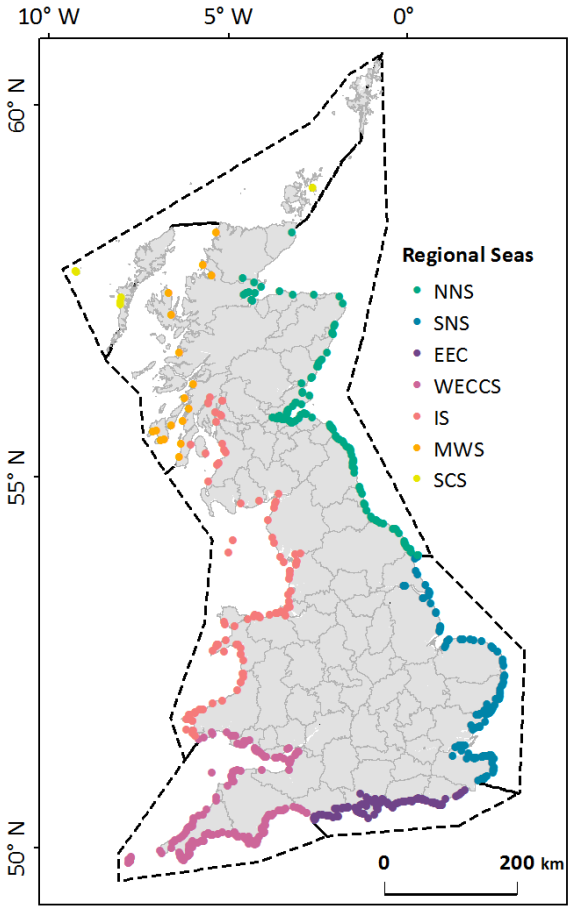


**Fig. 1.** Distribution of survey beaches – dashed line indicates Regional Sea boundary and coloured symbols correspond to relevant designation (green; NNS = Northern North Sea, blue; SNS = Southern North Sea, purple; EEC = Eastern English Channel, pink; WECCS = Western English Channel and Celtic Sea, coral; IS = Irish Sea, orange; MWS = Minches and West Scotland, yellow; SCS = Scottish Continental Shelf).

The number of beach litter surveys fluctuated annually and per month (recorded as counts of beaches surveyed per year from 2005-2014 and per month respectively; Fig. 2a and Fig. S1) and among regions (recorded as counts of surveys per Regional Sea across study period; Fig. 2b). The number of volunteer participants and duration of surveys also varied among years (recorded as counts of volunteers and hours spent surveying respectively per year from 2005-2014; Fig. 2c and d), as did the frequency of surveys per beach and intervals between surveys.

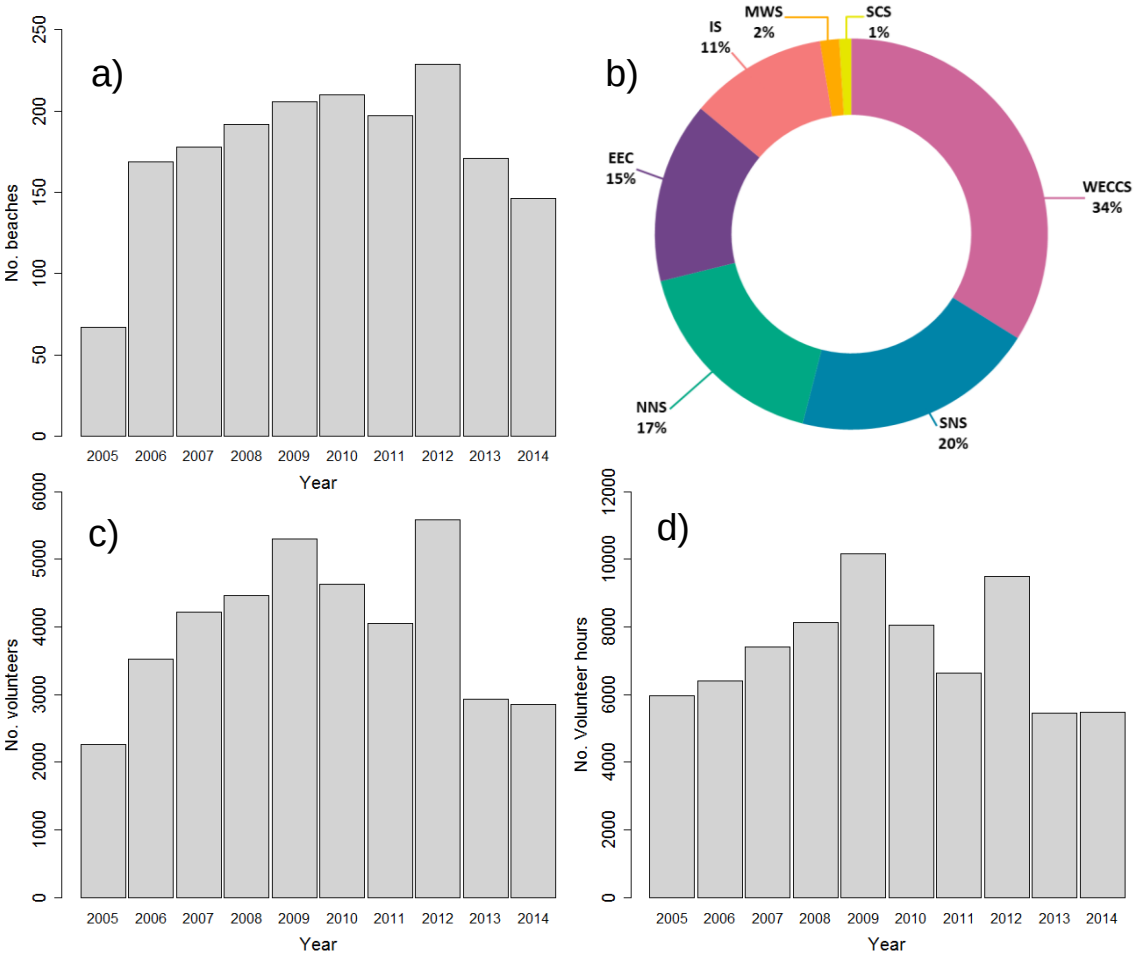


**Fig. 2.** Plots showing **a)** Number of beaches surveyed for litter per year between 2005 and 2014 (of *n* = 736 investigated); **b)** Proportion of effort (number of surveys) per Regional Sea (WECCS = Western English Channel and Celtic Sea, 34%; SNS = Southern North Sea, 20%; NNS = Northern North Sea, 17%; EEC = Eastern English Channel, 15%; IS = Irish Sea, 11%; MWS = Minches and West Scotland, 2%; SCS = Scottish Continental Shelf, 1%) **c)** Number of volunteer participants per year between 2005 and 2014; **d)** Number of volunteer hours spent surveying per year between 2005 and 2014.

Survey best practice instructions indicated that a 100 m survey should be undertaken. Given the nature of the project, however, and the desire for volunteers to survey and clear longer stretches of beaches, surveys were frequently longer in distance. In addition, there was no prior standardisation of the number of volunteers or time spent searching (duration). These factors were recorded, however, allowing for the variation in effort among surveys to be calculated and subsequently used to standardise data gathered. The number of participants was variable (range: 1 - 945 people per survey, mean ± SD = 12.3 ± 22.4 people , median = 8 people, inter-quartile range (IQR) = 3 - 15 people) as was survey duration (range: 10 min – 8 hrs, mean ± SD = 1.71 ± 0.95 hrs, median = 1.5 hrs, IQR = 1 - 2 hrs) and survey distance covered (range: 1 m - 7.5 km, mean ± SD = 432 ± 662 m, median = 140 m, IQR = 100 - 500 m; see Supplemental Material Fig. S2.). Various methods of outlier removal were investigated but it was preferred that all data collected were utilised.

To collect the data, volunteers would walk between the back of the beach and the strand-line, loosely adhering to a linear transect (parallel to the strand-line), searching for litter. Litter identification guides were provided to ensure accurate recording of items by volunteers. In addition, face to face training was offered to beach survey organisers, enabling them to support the volunteers in following the protocol. Gathered items of litter were assigned to one of 101 item categories that could be further classified into 12 material groups (plastic, polystyrene, rubber, cloth, metal, medical, sanitary, faeces, paper, wood, glass, pottery/ ceramic; see Supplemental Material Table S1). These classifications were pre-determined by MCS. Upon completion of a survey, all anthropogenic litter items recorded were summed, validated by a survey coordinator and subjected to further quality control by MCS. All collected litter items were removed from the beach.

*Data preparation and effort correction*

Significant linear relationships were determined between the number of litter items surveyed and three variables relating to effort (linear model(s): distance ($F_{1, 3058} = 8.6491$, $p=0.003$); duration ($F_{1, 3058} = 165$, $p=< 0.001$); number of volunteers ($F_{1, 3058} = 634$, $p=< 0.001$)). Data (i.e. counts of items) were standardised to account for variations in effort among beach litter surveys using Eq. 1; where *C*

= total count (no. items); *L* = survey linear distance (m); *D* = survey duration (mins); *V* = number of volunteers (people):

$$Eq.\ 1.\ \ A = \frac{C}{L(DV)}$$

The unit of the adjusted count (*A*) was *items collected per metre per minute per person* (number of items $m^{-1}$ $min^{-1}$ $person^{-1}$). It was therefore possible to investigate differences in litter density among beaches irrespective of varying volunteer effort.

*Descriptive statistics*

Using our standardised counts (number of items $m^{-1}$ $min^{-1}$ $person^{-1}$), the proportion (as number of items independent of mass or volume) of each litter item category (*n* = 101) and material group (*n* = 12) was calculated for all survey events and for each Regional Sea area. Where possible, items were attributed to a pathway (non-sourced, public litter, fishing, sewage, shipping, fly-tipped, medical) based on MCS classifications (see Supplemental Material Table S2) and, where possible, assigned as originating from either land- or marine-based activities (see Supplemental Material Table S3). Where litter items could not be assigned to either of these origin groups they were deemed non-sourced.

*Spatial analyses*

For each beach and Regional Sea area, the mean number of items $m^{-1}$ $min^{-1}$ $person^{-1}$ across the study period (2005-2014) was calculated for total litter and three types of litter of interest - food and drink packaging, fishing gear and wet wipes, chosen as they represent the three major pathways – public littering, fishing and sewage respectively. The former two types are assemblages of related items, whereas wet wipes are a stand-alone individual item category (see Supplemental Material Table S4). Beaches and regions were then ranked based on their mean standardised count values, from high to low. Annual mean estimates of standardised counts (for total litter) were also subject to spatial analysis using Moran's I clustering in ArcMap 10.2.2 (ESRI, 2014) – a technique which identifies statistically significant areas of litter presence and absence.

*Temporal analyses*

Generalised Linear Mixed Models (GLMMs) were used to examine temporal patterns in the abundance of total litter (standardised counts for all beaches), individual item categories (20 most common plus three additional item categories of interest). Analyses were undertaken in the statistical computing software, R (GLMM; 'lme4' package for R; R Development Core Team, 2015). Beach-specific identification numbers were used as a random effect in the model to account for the variation in survey frequency among beaches. *Season* and *region* were incorporated within the GLMM as fixed effects in addition to *year*. The normality of the dependent variable was assessed using a Q-Q plot and determined to be non-normal. As such, the data were log-transformed (log10) and further assessed using a second Q-Q, which confirmed a satisfactory transformation ('car' and 'MASS' packages for R; R development Core Team, 2015). Statistical significance was set at a probability level (α) of 0.05. To deal with multiple testing of individual item categories ($n$ = 23), a Bonferroni correction was applied and the probability threshold adjusted to < 0.0021 ($\alpha/n$). Seasons were defined as; *spring* (March, April, May), *summer* (June, July, August), *autumn* (September, October, November), *winter* (December, January, February).

## Results

*Descriptive statistics*

Anthropogenic litter was recorded during all beach litter surveys ($n$ = 3245) and a total of 2,376,541 items were collected from 1,402 km of cumulative surveyed coastline, with volunteers contributing 73,167 hours (equivalent to ~25 years of continuous surveying (365 days a year) by a single person working eight hours per day). Mean abundance across all beaches was 0.0085 items $m^{-1}$ $min^{-1}$ $person^{-1}$, with a maximum of 0.3297 items $m^{-1}$ $min^{-1}$ $person^{-1}$. This is equivalent to 51 items and 1978 items respectively, based on a survey carried out over 100 m for one hour by one volunteer. Large plastic fragments (>25 mm) was the most frequently recorded item category, representing 13% of all litter items, followed by small plastic fragments (<25 mm) at 10% (Table 1 for 20 most common item categories).

**Table 1.** Twenty (of *n* = 101) most common litter item categories recorded on British beaches (2005 – 2014 inclusive), by proportion.

| Item category | Proportion |
|---|---|
| Plastic fragments (large; >2.5cm) | 0.13 |
| Plastic fragments (small; <2.5cm) | 0.10 |
| Plastic caps | 0.07 |
| Polystyrene (small; <50cm) | 0.07 |
| Crisp packets | 0.06 |
| Fishing net (small; <50cm) | 0.05 |
| Plastic string | 0.05 |
| Plastic drinks bottles | 0.04 |
| Cotton buds | 0.03 |
| Fishing line | 0.03 |
| Cigarette stubs | 0.03 |
| Plastic cutlery | 0.02 |
| Glass fragments | 0.02 |
| Cloth pieces | 0.02 |
| Plastic bags | 0.02 |
| Polystyrene foam | 0.02 |
| Metal Drinks can | 0.02 |
| Plastic rope | 0.01 |
| Fishing net (large; >50cm) | 0.01 |
| Wood pieces | 0.01 |

Of the 12 material groups, plastic was the most dominant (66%), with expanded polystyrene and sanitary items representing 10% and 5% respectively (Fig. 3a).

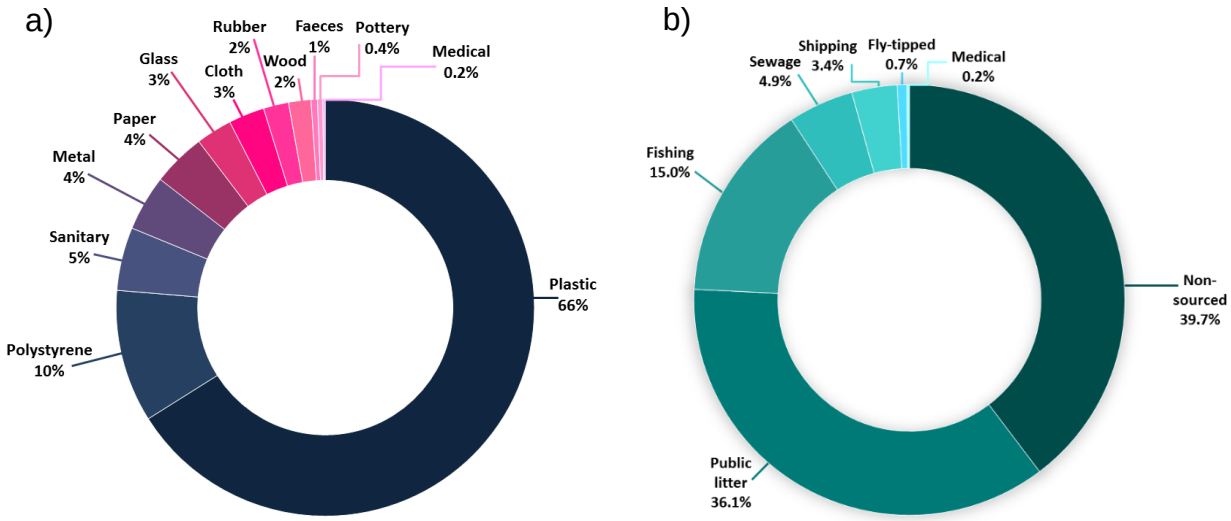


**Fig. 3** Composition of marine anthropogenic litter across all beaches surveyed as proportions by **a)** material (plastic; 66%, polystyrene; 10%, sanitary; 5%, medical; 4%, paper; 4%, glass; 3%, cloth; 3%, rubber; 2%, wood; 2%, faeces; 1%, pottery; 0.4% and medical; 0.2%) and **b)** pathway (non-sourced; 39.7%, public litter; 36.1%, fishing; 15.0%, sewage; 4.9%, shipping; 3.4%, fly tipped; 0.7% and medical; 0.2%).

The Scottish Continental Shelf (SCS) exhibited the highest proportion of plastic (83%) in beach litter surveys while the neighbouring region of Minches and West Scotland (MWS) exhibited the lowest (52%; Fig. 4a). The Northern North Sea (NNS) experienced the highest proportion of polystyrene (14%) and sanitary items (7%; Fig. 4b and c). In contrast, the Scottish Continental Shelf region reported the lowest proportions for both (3% and 0.2% respectively; Fig. 4b and c).

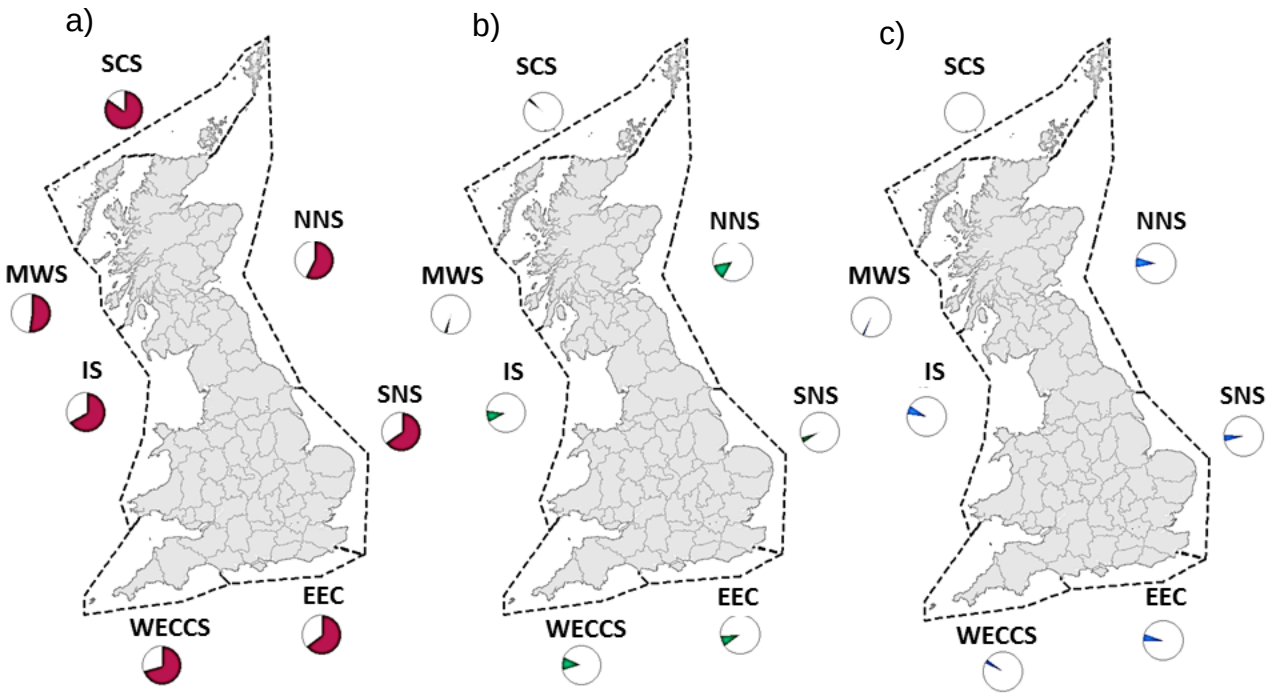


**Fig. 4.** Regional proportions of three most collected materials **a)** Plastic **b)** Polystyrene **c)** Sanitary. NNS = Northern North Sea; SNS = Southern North Sea; EEC = Eastern English Channel; WECCS = Western English Channel and Celtic Sea; IS = Irish Sea; MWS = Minches and West Scotland; SCS = Scottish Continental Shelf.

After non-sourced items (40%), public littering represented the most common pathway (36%), followed by fishing (15%), sewage (5%), shipping (3%), fly-tipping (0.7%) and medical (0.2%; Fig. 3b). Of items that could be attributed to an origin, 42% derived from land-based sources, such as littering (e.g. food packaging) and sewage (e.g. sanitary items), and 18% from marine-based activities, such as fishing and shipping. The remaining 40% consisted of items that could not be definitively assigned to either source category (e.g. fragments of various materials and generic items whose origin could either be from land- or marine-based sources). The Southern North Sea, Northern North Sea and Irish Sea encountered the highest proportion of litter from land-based activities (50%) and the Scottish Continental Shelf the lowest (20%; Fig. 5a.). This region (SCS) experienced the greatest proportion of litter attributed to marine-based activities (40%; Fig. 5b). There was little variation in the proportion of non-sourced items among the regions (35-40%; Fig. 5c).

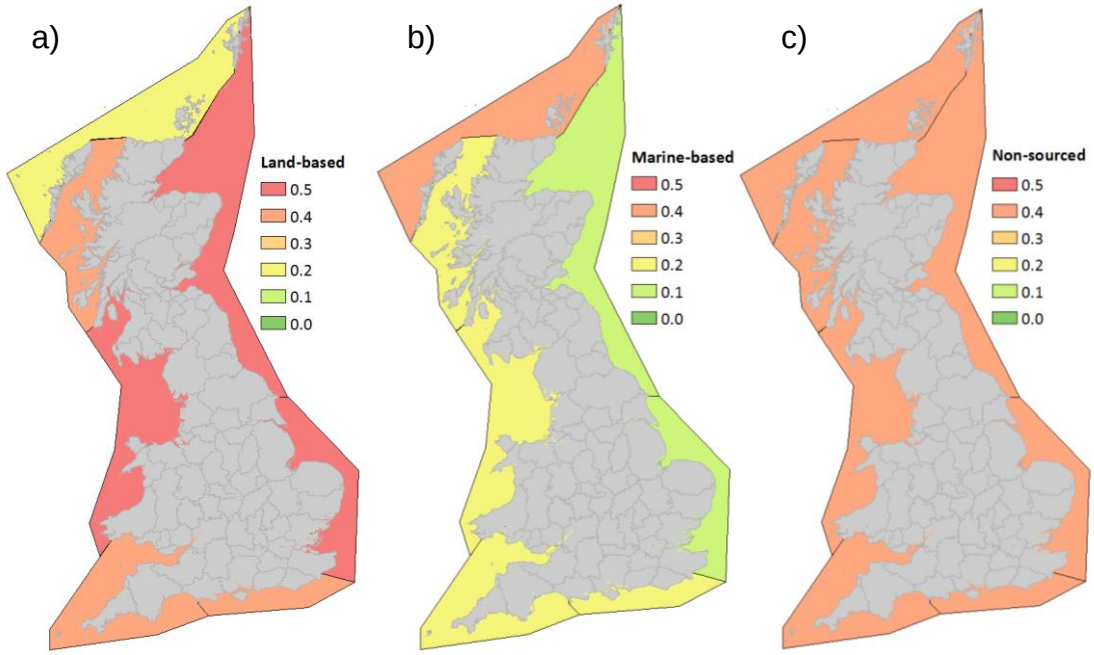


**Fig. 5**. Distribution-maps showing regional proportions (red = 0.5, orange = 0.4, gold = 0.3, yellow = 0.2, light green = 0.1, dark green = 0.0) of litter from **a)** land-based activities **b)** marine-based activities and **c)** non-sourced items.

*Spatial analyses*

The five most affected beaches (mean number of items $m^{-1}$ $min^{-1}$ $person^{-1}$ > 0.1) were heterogeneously distributed across Britain within four of the seven Regional Seas. Clustering analysis (Moran's I) revealed five areas where adjacent beaches share similar high levels of litter abundance, in Kent, Hampshire, Cornwall and the Bristol Channel (Lundy Island; Supplemental Material Fig. S3). Variations in regional mean abundances were evident, indicating significant differences among the Regional Seas (one-way ANOVA, $F_{6,\ 3238} = 37.95$, $p<0.001$; Fig. 6).

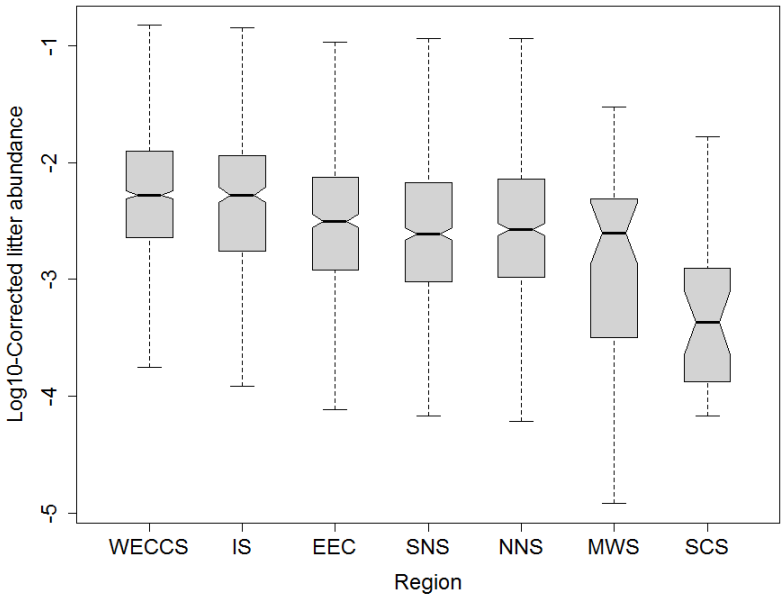


**Fig. 6.** Regional differences in log corrected litter abundance (WECCS = Western English Channel and Celtic Sea; IS = Irish Sea; EEC = Eastern English Channel; SNS = Southern North Sea; NNS = Northern North Sea; MWS = Minches and West Scotland; SCS = Scottish Continental Shelf). The thick black lines inside the notches (95% confidence interval of median) represent median values, grey boxes depict first and third quartiles and the whiskers illustrate the minimum and maximum values.

The Western English Channel and Celtic Sea exhibited the greatest mean abundance of 0.012 items $m^{-1}$ $min^{-1}$ $person^{-1}$ while the Scottish Continental Shelf exhibited the smallest of 0.002 items $m^{-1}$ $min^{-1}$ $person^{-1}$ (Fig. 7a). The Western English Channel and Celtic Sea exhibited the highest mean abundance of both food and drink packaging and fishing gear (0.0027 and 0.0015 and items $m^{-1}$ $min^{-1}$ $person^{-1}$ respectively; Fig 7b and c). The Southern North Sea exhibited the highest mean abundance of wet wipes (0.0001 items $m^{-1}$ $min^{-1}$ $person^{-1}$; Fig. 7d)

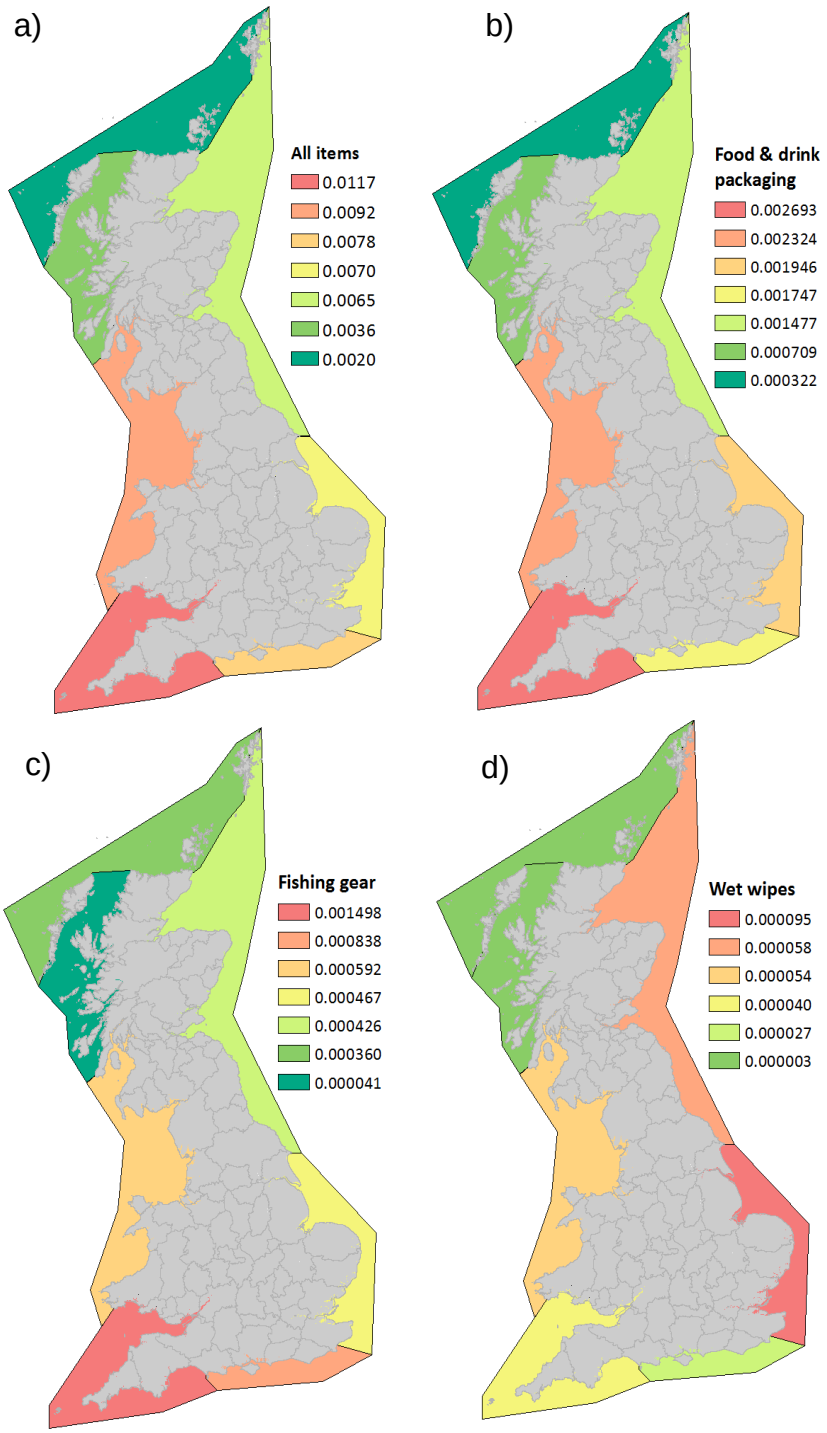


**Fig. 7.** Distribution-maps of regional mean number of items $m^{-1}$ $min^{-1}$ $person^{-1}$ (dark green to red = low to high) for **a)** all litter items **b)** food and drink packaging **c)** fishing gear **d)** wet wipes.

*Temporal analyses*

*Seasonal variation*

The overall abundance of litter was not significantly affected by season (one-way ANOVA, $F_{3, 3241} = 1.21$, p=>0.05). Nor was there a significant seasonal effect on

the abundance of litter from land-based sources (one-way ANOVA, $F_{3, 3241} = 0.13$, p=>0.05) or marine-based sources (one-way ANOVA, $F_{3, 3241} = 1.13$, p=>0.05).

*Long-term trends*

Analysis of the long-term trends using GLMMs indicated that the standardised litter abundance (number items $m^{-1}$ $min^{-1}$ $person^{-1}$) did not change significantly over the study period (2005-2014); removing Year from the model had no significant effect, *p*-value = 0.39. This analysis was repeated to investigate long-term trends in abundance of the 20 most common item categories as well as balloons, wet wipes and plastic food packaging due to concerns for their environmental impact. Six of these items experienced a significant increase - small plastic fragments (2.3 fold; i.e. from 0.00011 to 0.00037 number items $m^{-1}$ $min^{-1}$ $person^{-1}$ over 10 years); plastic food packaging (1.0 fold); wet wipes (0.9 fold); polystyrene foam (0.7 fold); balloons (0.6 fold); large fishing net (0.5 fold) - while the remaining items exhibited no significant temporal trend (Table 2).

**Table 2.** Results of long-term trend analysis using Generalised Linear Mixed Models (GLMMs) for top 20 (by proportion) individual litter items plus balloons, wet wipes and plastic food packaging based on mean across all surveys.

| Item | *p*-value (α) | Standard Error | t value | *p*-value accepted following Bonferroni adjustment to significance threshold | Fold Change |
|---|---|---|---|---|---|
| Plastic fragments (large; >2.5cm) | 0.0048 | -- | -- | N | -- |
| Plastic fragments (small; | <0.001 | 0.005581 | 10.37 | Y | + 2.3 |
| Plastic caps | 0.9472 | -- | -- | N | -- |
| Polystyrene (small; <50cm) | 0.5235 | -- | -- | N | -- |
| Crisp packets | 0.7782 | -- | -- | N | -- |
| Fishing net (small; <50cm) | 0.8307 | -- | -- | N | -- |
| Plastic string | 0.5947 | -- | -- | N | -- |
| Plastic drinks bottles | 0.1279 | | | N | -- |
| Cotton bud sticks | 0.0781 | -- | -- | N | -- |
| Fishing line | 0.3836 | -- | -- | N | -- |
| Cigarette stubs | 0.0507 | -- | -- | N | -- |
| Plastic cutlery | 0.1959 | -- | -- | N | -- |
| Glass fragments | 0.0800 | -- | -- | N | -- |
| Cloth pieces | 0.0027 | -- | -- | N | -- |
| Plastic bags | 0.5031 | -- | -- | N | -- |
| Polystyrene foam | 0.0002 | 0.005993 | 3.703 | Y | + 0.7 |
| Metal Drinks can | 0.6405 | -- | -- | N | -- |
| Plastic rope | 0.3550 | -- | -- | N | -- |
| Fishing net (large; >50cm) | 0.0019 | 0.007563 | 3.097 | Y | + 0.5 |
| Wood pieces | 0.4704 | -- | -- | N | -- |
| Balloons | 0.0005 | 0.005942 | 3.460 | Y | + 0.6 |
| Wet wipes | 0.0001 | 0.008088 | 3.819 | Y | + 0.9 |
| Plastic food packaging | <0.001 | 0.005856 | 5.545 | Y | + 1.0 |

## Discussion

*Descriptive statistics*

Given their durability, it is perhaps unsurprising that items made from synthetic materials comprise a large proportion of anthropogenic litter. Large and small plastic fragments are generated by the degradation of larger items, and so they represent the accumulated remains of many years of waste. They will be broken down further by UV photo-degradation and wave action until they become micro-plastics, small synthetic particles that can be ingested by a range of organisms, including zooplankton, commercial fish species and other sea foods consumed by humans, and marine megafauna (Besseling et al., 2015; Cole et al., 2013; Neves et al., 2015; Rochman et al., 2015). The Scottish Continental Shelf experienced the highest proportion of plastic whilst its neighbouring region, Minches and West Scotland exhibited the lowest. Due to its remote location, it is likely that the former is exposed to inputs from fairly uniform sources, mainly fisheries and floating debris from other countries within the north Atlantic. This is further highlighted by the fact that the region (SCS) also exhibited the greatest proportion of litter attributed to marine-based activities. Over a third of total litter originates from public littering, indicating that land-based inputs are likely key sources of marine anthropogenic litter. These results correspond with those from previous studies in other areas, such as the Mediterranean Sea, the Great Lakes (USA) and the SE Pacific, though the proportions vary (Bravo et al., 2009; Hidalgo-Ruz and Thiel, 2013; Hoellein et al., 2015; Munari et al., 2015; Topçu et al., 2013).

*Spatial patterns*

Although the most affected beaches were heterogeneously distributed across Britain, there were strong differences among the regions and the Western English Channel and Celtic Sea exhibited the highest mean abundance of litter from both land and sea. This may be due to a number of reasons, such as the presence of large cities and discharging rivers (Swansea, Cardiff, Newport, Bristol, Plymouth; River Severn), high levels of fishing effort (Lee et al., 2010; Witt and Godley, 2007), the world's third busiest shipping route - the English Channel - and input from the wider Atlantic Ocean (wind and currents). In addition, this region represents a popular tourist destination, particularly during the summer months. The south west of England attracts the highest number of domestic tourists of all

UK regions (Smith, 2010) and it is estimated that approximately five million visits are made to Cornwall alone each year (South West Research Company, 2010). This high density of beach-users likely contributes to the observed levels of anthropogenic litter.

*Temporal trends*

There was an absence of a temporal trend in the overall abundance of marine anthropogenic litter through the 10-year dataset. This lack of change may be due to a number of reasons. Firstly, the amount of litter may have indeed changed little over the 10-year period. Secondly, it may be that the time-period is insufficient to statistically reveal small changes within a variable system. For example, one study surmised that some sampling regimes are unlikely to detect a ≤ 30% change within 25 years but a 40% - 50% change may be detected in 10 - 15 years (Moriarty et al., 2016). Thirdly, it is possible that the methodological constraints, such as the need for effort correction, and variability within the system (due to the multitude of inputs and extensive transportation of debris by currents and wind) dilute the statistical signal (Ryan et al., 2009; Schulz et al., 2015). Finally, the extent of litter removal by volunteers and local authorities may be large enough to limit the accumulation of debris and effectively prevent its escalation (Hoellein et al., 2015), but insufficient to make detectable improvements. Further work is required to better understand these factors.

Temporal trends for some individual items were identified. The more than two-fold observed increase in small plastic fragments is likely a result of the perpetual break-down of larger plastic items by UV photo-degradation and wave action. As a result, the number of small plastic pieces is likely to rise exponentially into the future, especially given the current and predicted levels of plastic litter input to the marine environment. The increase in both balloons and large fishing net abundance is of concern due to the threat they pose to biodiversity, particularly seabirds, marine mammals and marine turtles, through ingestion and entanglement (Allen et al., 2012; da Silva Mendes et al., 2015; Plotkin et al., 1993). Though fishing gear is usually lost accidentally, balloons are often actively released *en masse* at public events and our results show a significant increase in the number recorded during surveys. Balloons are not currently defined as ‘litter’ under the UK Environmental Protection Act (EPA) 1990 whereby it is an offence to drop “or otherwise deposit” litter in a public place (Parliament of the

United Kingdom, 1990). Some local authorities, however, do recognise the threat posed by balloons and have voluntarily banned releases on their property. It would seem judicious that revisions are made to the EPA that reflect these concerns and legislatively prevent such mass littering events from occurring. Wet wipes may enter the marine environment via waste water from domestic sources. Many contain plastic and so persist indefinitely, often leading to blockages within sewerage systems. It is estimated that approximately £88 million is spent in the UK annually as a result (Water UK, pers. comm., 2016). The increase reflected in our results demonstrates an urgent need for mitigation. The observed increases in other items, such as polystyrene foam and plastic food packaging, illustrates the need for a reduction in their inappropriate disposal as well as biodegradable alternatives to such materials, e.g. cardboard.

*Recommendations for future work*

Citizen science projects are valuable in terms of their ability to generate large-scale data on the distribution and abundance of marine anthropogenic litter (Hidalgo-Ruz and Thiel, 2015, 2013). Yet, we acknowledge a number of constraints that are worthy of discussion and make recommendations for future work based on our findings. We recognise that implementing all of the recommended measures may not be logistically feasible for some beach litter programmes (due to factors such as, volunteer availability, health and safety, time and resources) but outline a series of measures based on a best-case scenario;

*Site selection*: Survey beaches were chosen by local volunteers and so it is possible that those perceived as 'dirty' or iconic, or of special environmental value (such as Sites of Special Scientific Interest; SSSIs) may be preferentially selected above other sites which have little or no debris (Browne et al., 2015). Logistical factors, such as beach accessibility and therefore ease of litter removal, may also be a selection factor. This inherent bias could be eliminated by employing a random sampling approach but would likely be constrained by volunteer availability, willingness of volunteers to visit less desirable sites and health and safety considerations at certain locations.

*Survey protocol:* Though data adjustment is a useful method of retrospectively correcting for variation in survey effort, the use of standardised survey protocol based on OSPAR's Guidelines is optimal (OSPAR, 2010) .In particular, efforts should be made to use the same sampling unit (repeated

sampling of same 100 m section of beach) for each survey as this would likely reduce variation within dataset. We also recommend that a standard number of volunteers (e.g. 2) survey the 100 m section for a set amount of time to ensure the degree of effort is consistent across surveys. Following this, any remaining litter may be removed using a non-standardised method. In addition, as some litter items may be less numerous but larger in size (i.e. fishing nets) it may advantageous to record item mass as well as frequency where possible. This would also enhance the potential to compare survey results with those of similar studies (Ryan et al., 2009) but likely be constrained by availability of resources.

*Area surveyed*: Although it was possible to adjust the data to account for variation in survey distance, the effective width of the transects was not always recorded and so the total area covered was unknown. Such information would enhance the reliability of abundance estimates and make comparisons among surveys more feasible. de Araújo et al., (2006) found that the diversity of item categories detected was related to sampling transect area and the number of categories significantly increased with transect width but stabilised from 15-20m onwards. As such, it would seem pertinent to standardise width or at the very least, record it so that retrospective adjustment can be applied.

*Disparities among volunteers*: Statistically, survey participants were treated uniformly, but in reality they likely differed in their personal effort and ability to search for, collect and categorise litter. These disparities may be affected by factors, such as age. For example, young children may present difficulties when distinguishing among the various material types, particularly for smaller items (Hidalgo-Ruz and Thiel, 2013). Illustrative guides are a useful tool for minimising this potential source of error (Eastman et al., 2014; Hidalgo-Ruz and Thiel, 2013). Further investigation is required to better understand how factors, such as age and gender, affect the types and amount of litter gathered and recorded. In addition, we recommend that survey leaders, where possible, undergo training prior to the event taking place as in Hoellein et al., (2015).

*Sightability bias*: Volunteers may be more or less likely to detect, gather and record certain items of litter due to known or subconscious preference. For example, items with a recognisable purpose, such as a plastic drinks bottle, may be more likely to be seen than generic items, such as fragments of plastic or pieces of glass. Quantitative methods, such as detectability trials whereby beach litter composition before and after cleaning is compared, are required to

investigate the presence of detectability bias and correct for it if necessary. In addition, marine anthropogenic litter items not easily detectable by the naked eye, such as microplastics, may be under-recorded.

*Accumulation rates and long-term trends:* The intervals between beach cleans, carried out either by NGOs or local authorities, were not standardised and so litter removal varied temporally (Hoellein et al., 2015). For example, depending on ownership, bathing beaches may be subjected to regular (daily or weekly) cleaning during the tourist season but receive little litter management during the winter months. As a result, it is likely that the detectability of re-accumulation rates, and therefore trends in overall abundance within our dataset, was diminished (Smith and Markic, 2013). For this reason, OSPAR (2010) guidelines state that monitoring beaches should 'ideally not be subject to any other litter collection activities'. Although frequent sampling of all beaches to monitor accumulation rates would not be feasible due to the considerable amount of effort and resources required, a sub-sample of indicator beaches could be rigorously examined to infer patterns within the wider system. This would involve an initial beach clean to remove all litter followed by regular sampling (e.g. once a month) to record and remove any new items, as suggested by Ryan et al. (2009). This type of fine-scale sampling can provide insights in to local patterns and cycles. For a more broad-scale impression, some beach litter survey programmes, such as the MCS *Great British Beach Clean*, opt to survey at the same time each year. This method enhances inter-annual comparability and would be more sensitive in generating insights into long-term trends. Information on the rates of litter removal by local authorities and other bodies would further enhance understanding of re-accumulation.

*Origins and pathways:* In our study we were able to broadly assign litter items to originating from either land- or marine-derived sources based on their perceived original purpose. To better understand how litter arrives on beaches, it would be useful to differentiate between items that have previously entered the marine environment and re-stranded, and those directly deposited from land-based sources, for example, poor waste management or littering (Smith and Markic, 2013). Quantitative information on the various pathways could inform management recommendations and facilitate the development of measures to restrict the amount of litter entering the marine environment. For example, beaches that experience high levels of tourism, may also experience high

concentrations of items attributable to direct public littering. In such cases, efforts to increase awareness and provide appropriate and convenient waste disposal facilities may provide a suitable solution. Conversely, beaches with high use may experience lower levels of litter due to more frequent cleaning (Bravo et al., 2009). For monitoring purposes, we recommend that beach litter recording forms include the facility to document which pathway - directly deposited or re-stranded having spent time at sea – each item has taken. Pictorial guidance notes may assist volunteers in allocating items to the appropriate pathway. This may be constrained by the willingness of volunteers to undertake surveys once they reach a certain level of complexity and effort, as well as the ability to offer training to maintain consistency of recording of pathways.

*Value of citizen science*

The data analysed in this study were collected by volunteers of varying age and background, including school children and community groups. Their involvement as citizen-scientists is of considerable value; firstly, it enabled the removal of over two million (2,376,541) items of anthropogenic litter from British beaches. Second, it greatly reduced the cost of sampling. For example, if every volunteer hour (total=73,167) was charged at National Living Wage (£7.20 as of 1 April 2016; UK), data collection would have cost ~ £500,000 in salaries. Thirdly, activities such as beach cleans and litter surveys can enhance public appreciation of environmental issues, potentially leading to positive changes in behaviours and attitudes (Wyles et al., 2016). This is particularly important given that social viewpoints have a significant impact on littering behaviour and the acceptance of measures to reduce it (UNEP, 2016). Beach cleans are also associated with higher levels of marine awareness, demonstrating their educational value (Wyles et al., 2016). Lastly and crucially, citizen science programmes can also be instrumental in the generation of large, insightful datasets with broad temporal and spatial coverage - we analysed data collected by MCS volunteers during beach litter surveys in every month of the year for 10 years, around much of the British coastline.

## Conclusion

In summary, our results demonstrate how organised citizen science programmes that adopt a defined sampling approach and record effort can be effective for

monitoring marine anthropogenic litter. Volunteer-led beach cleans and litter surveys facilitate the removal of large quantities of litter from marine and coastal environments, reduce the cost of sampling, enhance public awareness of environmental issues and generate insightful data, all of which are necessary for addressing the complex problem of marine anthropogenic litter pollution. Ultimately though, the most efficacious and economic solution is to minimise and eventually prevent the release of anthropogenic waste into the marine environment by reducing our consumption and inappropriate disposal of synthetic and persistent materials, such as plastic.

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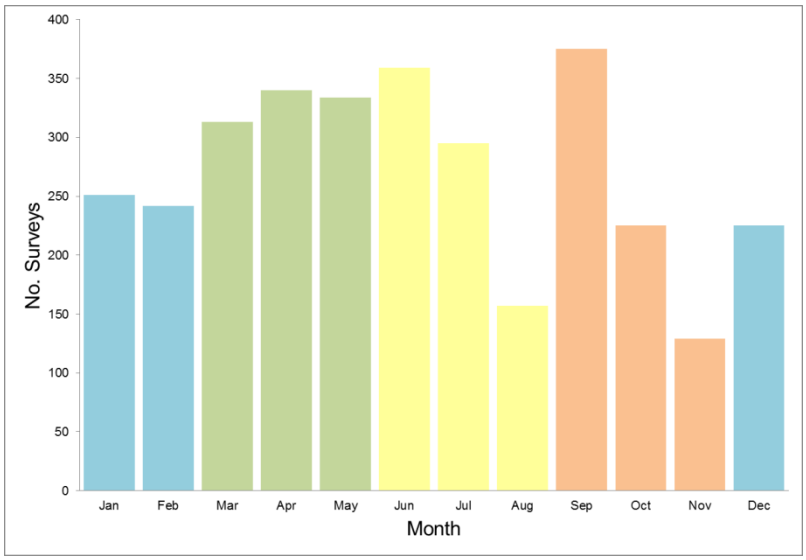
## Supplemental Information

**Fig. S1** Number of surveys per month that took place during the sampling period (2005 – 2014 inclusive; total *n* = 3245). Colour of bars indicate seasons – winter (blue), spring (green), summer (yellow), autumn (orange).



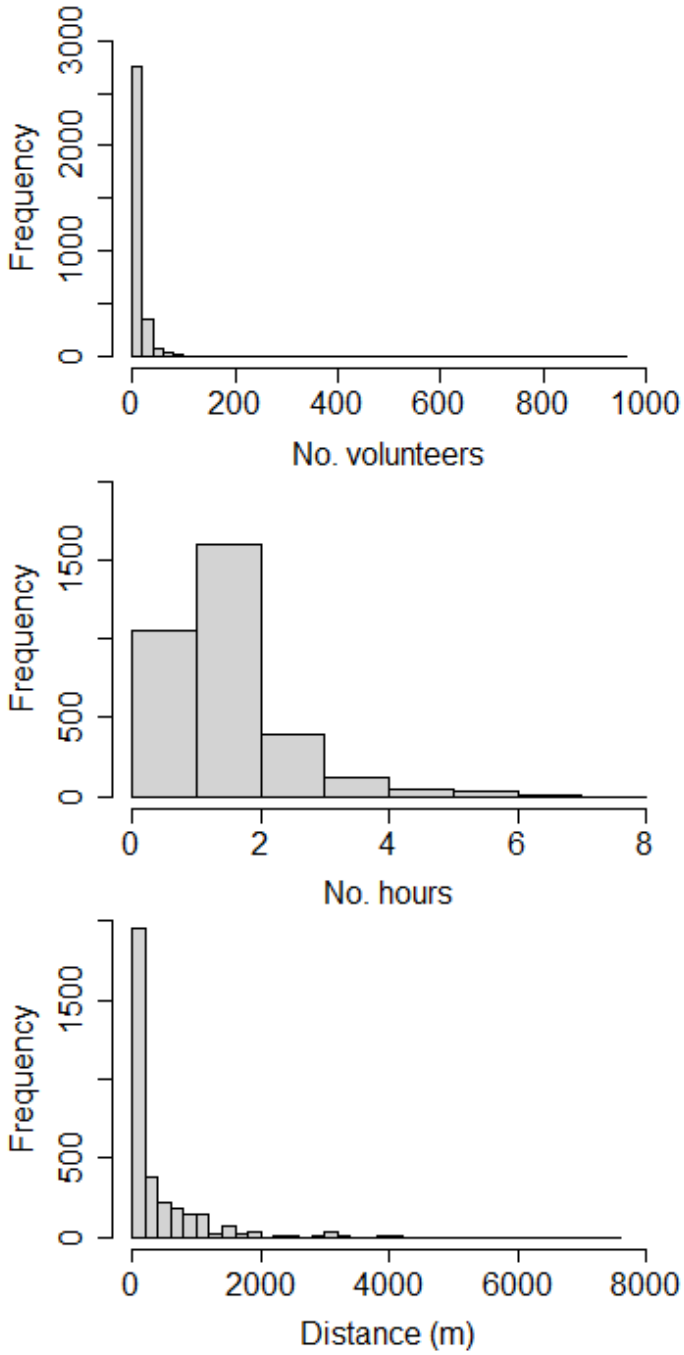



**Fig. S2** Frequency histograms of **a)** number of volunteers (range: 1 - 945 people per survey) taking part in a litter survey event **b)** number of hours (range: 10 min – 8 hrs) invested in a litter survey **c)** distance (m; range: 1 – 7500 m) covered for a litter survey.

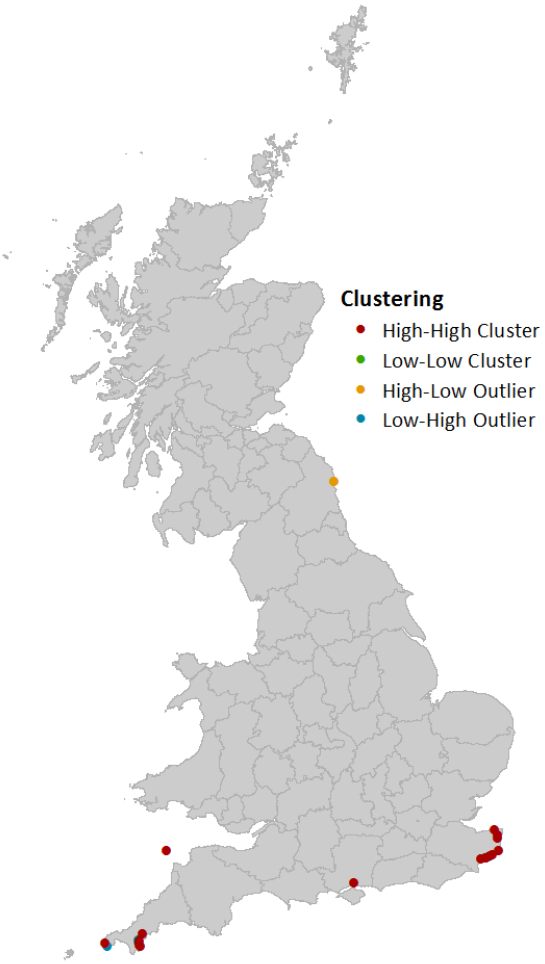



**Fig. S3.** Moran's I clustering revealed four main groups of localised high (red points) litter abundance 'hotspots' - in Kent, Hampshire, Cornwall and the Bristol Channel (Lundy Island).

**Table S1.** MCS recording form - Gathered items of litter were assigned to one of 101 categories that could be further classified into 12 material groups (plastic, polystyrene, rubber, cloth, metal, medical, sanitary, faeces, paper, wood, glass, pottery/ ceramic).

## Where does it all come from?

This list is FOR REFERENCE ONLY to help you see where all this litter comes from.

marine conservation society

| Plastic | total number |
|---|---|
| 4/6 pack yokes | PUBLIC LITTER |
| Bags (including supermarket) | PUBLIC LITTER |
| **Bottles, containers and drums** | |
| - Drinks | PUBLIC LITTER |
| - Cleaner | SHIPPING |
| - Food (e.g. pots, tubs, sachets) | PUBLIC LITTER |
| - Foreign | SHIPPING |
| - Oil | SHIPPING |
| - Toiletries | PUBLIC LITTER |
| Caps / lids | PUBLIC LITTER |
| Cigarette lighters / tobacco pouches | PUBLIC LITTER |
| Combs / hair brushes / sunglasses | PUBLIC LITTER |
| Crisp / sweet / lolly / sandwich wrappers | PUBLIC LITTER |
| Cutlery / trays / straws / cups | PUBLIC LITTER |
| Fish boxes | FISHING |
| Fishing line (anglers) | FISHING |
| Fishing net & net pieces < 50 cm | FISHING |
| Fishing net & net pieces > 50 cm | FISHING |
| Floats (fishing buoys) / reels | FISHING |
| Industrial packaging / crates / sheeting | SHIPPING |
| Lobster / crab pots & tops | FISHING |
| Mesh bags (eg. vegetable) | SHIPPING |
| Pens | PUBLIC LITTER |
| Rope diameter >1cm | SHIPPING |
| String & cord diameter <1cm | FISHING |
| Shoes / sandals | PUBLIC LITTER |
| Shotgun cartridges | PUBLIC LITTER |
| Strapping bands | SHIPPING |
| Toys / party poppers / fireworks / dummies | PUBLIC LITTER |
| Traffic cones | FLY-TIPPED |
| Plastic pieces < 2.5 cm | NON-SOURCED |
| Plastic pieces > 2.5 cm | NON-SOURCED |
| Other (specify) | NON-SOURCED |

| Polystyrene | |
|---|---|
| Buoys | FISHING |
| Fast food containers / cups | PUBLIC LITTER |
| Fish boxes | FISHING |
| Fibreglass | NON-SOURCED |
| Foam / sponge / insulation | NON-SOURCED |
| Packaging | NON-SOURCED |
| Polystyrene pieces < 50 cm | NON-SOURCED |
| Other (specify) | NON-SOURCED |

| Rubber | |
|---|---|
| Balloons / balloon string | PUBLIC LITTER |
| Boots | FISHING |
| Gloves (heavy duty) | FISHING |
| Gloves (light weight) | NON-SOURCED |
| Rubber pieces < 50 cm | NON-SOURCED |
| Tyres without holes / wheels | FLY-TIPPED |
| Tyres with holes | FISHING |
| Other (specify) | NON-SOURCED |

| Cloth | |
|---|---|
| Cloth pieces | NON-SOURCED |
| Clothing / shoes / beach towels | PUBLIC LITTER |
| Furnishings | FLY-TIPPED |
| Sacking | NON-SOURCED |
| Other (specify) | NON-SOURCED |

| Metal | total number |
|---|---|
| Aerosol cans | SHIPPING |
| BBQs (disposable) | PUBLIC LITTER |
| Bottle caps | PUBLIC LITTER |
| Car parts / car batteries | FLY-TIPPED |
| Drink cans | PUBLIC LITTER |
| Fishing weights / hooks / lures | FISHING |
| Foil wrappers | PUBLIC LITTER |
| Food cans | SHIPPING |
| Lobster / crab pots & tops | FISHING |
| Oil drums | SHIPPING |
| Scrap / metal appliances / paint tins | FLY-TIPPED |
| Household batteries | PUBLIC LITTER |
| Wire / wire mesh / metal pieces | NON-SOURCED |
| Other (specify) | NON-SOURCED |

| Medical | |
|---|---|
| Inhaler | MEDICAL |
| Plasters | MEDICAL |
| Syringes | MEDICAL |
| Other (specify) | MEDICAL |

| Sanitary | |
|---|---|
| Condoms | SEWAGE RELATED DEBRIS |
| Cotton bud sticks | SEWAGE RELATED DEBRIS |
| Nappies | SEWAGE RELATED DEBRIS |
| Tampon applicators / tampons | SEWAGE RELATED DEBRIS |
| Toilet fresheners | SEWAGE RELATED DEBRIS |
| Towels / panty liners / backing strips | SEWAGE RELATED DEBRIS |
| Wet wipes | SEWAGE RELATED DEBRIS |
| Other (specify) | SEWAGE RELATED DEBRIS |

| Animal faeces | Don't touch! |
|---|---|
| In bags | PUBLIC LITTER |
| Not in bags | PUBLIC LITTER |

| Paper | |
|---|---|
| Bags | PUBLIC LITTER |
| Cardboard | NON-SOURCED |
| Carton / purepak (e.g. milk) | PUBLIC LITTER |
| Carton / tetrapack (e.g fruit juice) | PUBLIC LITTER |
| Cigarette packets | PUBLIC LITTER |
| Cigarette stubs | PUBLIC LITTER |
| Cups | PUBLIC LITTER |
| Newspapers / magazines | PUBLIC LITTER |
| Other (specify) | NON-SOURCED |

| Wood | |
|---|---|
| Corks | PUBLIC LITTER |
| Lobster / crab pots & tops | FISHING |
| Pallets / crates | SHIPPING |
| Ice lolly sticks / chip forks | PUBLIC LITTER |
| Paint brushes | NON-SOURCED |
| Wood pieces (not twigs etc.) | NON-SOURCED |
| Other (specify) | NON-SOURCED |

| Glass | |
|---|---|
| Bottles | PUBLIC LITTER |
| Light bulbs / tubes | SHIPPING |
| Glass pieces | PUBLIC LITTER |

| Pottery/ceramic | |
|---|---|
| Any pottery or ceramic | FLY-TIPPED |

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**Table S2.** Items attributed to each Pathway (non-sourced, public litter, fishing, sewage, shipping, fly-tipped and medical)

| Pathway | | | | | | |
|---|---|---|---|---|---|---|
| **Non-sourced** | **Public litter** | **Fishing** | **Sewage** | **Shipping** | **Fly-tipped** | **Medical** |
| Cloth: Cloth | Cloth: Clothing | Metal: Fishing | San: Buds | Glass: Bulbs | Cloth: Furnishings | Med: Inhalers |
| Cloth: Other | Faeces: In bags | Metal: Lobsterpots | San: Condoms | Metal: Aerosol | Metal: Batteries | Med: Other |
| Cloth: Sacking | Faeces: Not bags | Plastic: Fishboxes | San: Nappies | Metal: Food | Metal: Car | Med: Plasters |
| Metal: Other | Glass: Bottles | Plastic: Fishing line | San: Other | Metal: Oil | Metal: Scrap | Med: Syringes |
| Metal: Wire | Glass: Glass | Plastic: Fishing net large | San: Tampons | Paper: Purepak | Plastic: Cones | |
| Paper: Cardboard | Metal: Bbqs | Plastic: Fishing net small | San: Toilet | Plastic: Cleaner | Pottery: Ceramic | |
| Paper: Other | Metal: Caps | Plastic: Floats | San: Towels | Plastic: Foreign | Rubber: Tyres | |
| Plastic: Other | Metal: Drink | Plastic: Lobsterpots | San: Wipes | Plastic: Industrial | | |
| Plastic: Plastic large | Metal: Foil | Plastic: String | | Plastic: Meshbags | | |
| Plastic: Plastic small | Paper: Bags | Poly: Buoys | | Plastic: Oil | | |
| Poly: Fibreglass | Paper: Cig packets | Poly: Fishboxes | | Plastic: Rope | | |
| Poly: Foam | Paper: Cig stubs | Rubber: Boots | | Plastic: Strapping | | |
| Poly: Other | Paper: Cups | Rubber: Gloves heavy | | Wood: Pallets | | |
| Poly: Packaging | Paper: Newspapers | Rubber: Tyres holes | | | | |
| Poly: Poly small | Paper: Tetrapak | Wood: Lobsterpots | | | | |
| Rubber: Gloves light | Plastic: Bags | | | | | |
| Rubber: Other | Plastic: Caps | | | | | |
| Rubber: Rubber small | Plastic: | | | | | |
| Wood: Brushes | Plastic: Combs | | | | | |
| Wood: Other | Plastic: Crisp | | | | | |
| Wood: Wood | Plastic: Cutlery | | | | | |
| | Plastic: Drinks | | | | | |
| | Plastic: Food | | | | | |
| | Plastic: Pens | | | | | |
| | Plastic: Shoes | | | | | |
| | Plastic: Shotgun | | | | | |
| | Plastic: Toiletries | | | | | |
| | Plastic: Toys | | | | | |
| | Plastic: Yokes | | | | | |
| | Poly: Food | | | | | |
| | Rubber: Balloons | | | | | |
| | Wood: Corks | | | | | |
| | Wood: Lolly | | | | | |

**Table S3.** Items attributed to originating from either land- or marine-based activities (or non-sourced).

| Origin | | |
|---|---|---|
| **Land** | **Marine** | **Non-sourced** |
| Cloth: Clothing | Glass: Bulbs | Cloth: Cloth |
| Cloth: Furnishings | Metal: Aerosol | Cloth: Other |
| Faeces: In bags | Metal: Fishing | Cloth: Sacking |
| Faeces: Not bags | Metal: Food | Metal: Other |
| Glass: Bottles | Metal: Lobsterpots | Metal: Wire |
| Glass: Glass | Metal: Oil | Paper: Cardboard |
| Med: Inhalers | Paper: Purepak | Paper: Other |
| Med: Other | Plastic: Cleaner | Plastic: Other |
| Med: Plasters | Plastic: Fishboxes | Plastic: Plastic large |
| Med: Syringes | Plastic: Fishing line | Plastic: Plastic small |
| Metal: Batteries | Plastic: | Poly: Fibreglass |
| Metal: Bbqs | Plastic: | Poly: Foam |
| Metal: Caps | Plastic: Floats | Poly: Other |
| Metal: Car | Plastic: Foreign | Poly: Packaging |
| Metal: Drink | Plastic: Industrial | Poly: Poly small |
| Metal: Foil | Plastic: Lobsterpots | Rubber: Gloves light |
| Metal: Scrap | Plastic: Meshbags | Rubber: Other |
| Paper: Bags | Plastic: Oil | Rubber: Rubber small |
| Paper: Cig packets | Plastic: Rope | Wood: Brushes |
| Paper: Cig stubs | Plastic: Strapping | Wood: Other |
| Paper: Cups | Plastic: String | Wood: Wood |
| Paper: Newspapers | Poly: Buoys | |
| Paper: Tetrapak | Poly: Fishboxes | |
| Plastic: Bags | Rubber: Boots | |
| Plastic: Caps | Rubber: Gloves heavy | |
| Plastic: | Rubber: Tyres holes | |
| Plastic: Combs | Wood: Lobsterpots | |
| Plastic: Cones | Wood: Pallets | |
| Plastic: Crisp | | |
| Plastic: Cutlery | | |
| Plastic: Drinks | | |
| Plastic: Food | | |
| Plastic: Pens | | |
| Plastic: Shoes | | |
| Plastic: Shotgun | | |
| Plastic: Toiletries | | |
| Plastic: Toys | | |
| Plastic: Yokes | | |
| Poly: Food | | |
| Pottery: Ceramic | | |
| Rubber: Balloons | | |
| Rubber: Tyres | | |
| San: Buds | | |
| San: Condoms | | |
| San: Nappies | | |
| San: Other | | |
| San: Tampons | | |
| San: Toilet | | |
| San: Towels | | |
| San: Wipes | | |
| Wood: Corks | | |
| Wood: Lolly | | |

**Table S4.** Items attributed to Food & Drink packaging, Fishing Gear and Wet Wipes

| Type | | |
|---|---|---|
| **Food & drink** | **Fishing gear** | **Wet wipes** |
| Plastic: Drinks | Plastic: Fishboxes | San: Wipes |
| Plastic: Food | Plastic: Fishing_line | |
| Plastic: Caps | Plastic: Fishing_net_small | |
| Plastic: Crisp | Plastic: Fishing_net_large | |
| Plastic: Cutlery | Plastic: Lobsterpots | |
| Poly: Food | Metal: Fishing | |
| Metal: Bbqs | Metal: Lobsterpots | |
| Metal: Drink | Wood: Lobsterpots | |
| Metal: Food | | |
| Paper: Purepak | | |
| Paper: Tetrapak | | |
| Paper: Cups | | |
| Wood: Lolly | | |
| Glass: Bottles | | |

# Chapter 2: Investigating microplastic trophic transfer in marine top predators

This chapter is a reformatted copy of my publication: **Nelms SE**, Galloway TS, Godley BJ, Jarvis DS, Lindeque PK (2018). Investigating microplastic trophic transfer in marine top predators. *Environmental Pollution*. 238: 999-1007. I conducted all of the sample processing and analysis and was lead author on this work; DJ provided the samples; PKL guided the development of the project; all authors provided comments and edits to help shape the final manuscript. **Impact: Citations – 50; Altmetric score - 508**


## Abstract

Microplastics are highly bioavailable to marine organisms, either through direct ingestion, or indirectly by trophic transfer from contaminated prey. The latter has been observed for low-trophic level organisms in laboratory conditions, yet empirical evidence in high trophic-level taxa is lacking. *In natura* studies face difficulties when dealing with contamination and differentiating between directly and indirectly ingested microplastics. The ethical constraints of subjecting large organisms, such as marine mammals, to laboratory investigations hinder the resolution of these limitations. Here, these issues were resolved by analysing sub-samples of scat from captive grey seals (*Halichoerus grypus*) and whole digestive tracts of the wild-caught Atlantic mackerel (*Scomber scombrus*) they are fed upon. An enzymatic digestion protocol was employed to remove excess organic material and facilitate visual detection of synthetic particles without damaging them. Polymer type was confirmed using Fourier-Transform Infrared (FTIR) spectroscopy. Extensive contamination control measures were implemented throughout. Approximately half of scat subsamples (48%; $n$ = 15) and a third of fish (32%; $n$ = 10) contained 1 - 4 microplastics. Particles were mainly black, clear, red and blue in colour. Mean lengths were 1.5 mm and 2 mm in scats and fish respectively. Ethylene propylene was the most frequently detected polymer type in both. Our findings suggest trophic transfer represents an indirect, yet potentially major, pathway of microplastic ingestion for any species whose feeding ecology involves the consumption of whole prey, including humans.

## Introduction

Microplastics (< 5 mm in size) are ubiquitous in a wide range of marine habitats (GESAMP, 2015) and research interest is growing to better understand their impacts on the health of the marine environment and the organisms within it. These synthetic and persistent particles originate from a variety of sources, which include the fragmentation of larger macro-plastics (e.g. fishing gear, packaging) by UV photo-degradation, wave action and physical abrasion; shipping spills of pre-production pellets (nurdles) and polystyrene beads; the discharge of waste water containing microbeads used in cosmetics and microfibers released during the washing of textiles; and run-off from land containing road marking paint and vehicle tyre fragments (Andrady, 2011; Barnes et al., 2009; Boucher and Friot, 2017; Browne et al., 2011; Napper and Thompson, 2016; UNEP, 2009). Their small size means that microplastics are bioavailable to ingestion by a variety of taxa including zooplankton, marine invertebrates, fish, seabirds, and marine mammals (Amélineau et al., 2016; Cole et al., 2013; Lusher et al., 2015, 2013). Reasons for *direct* ingestion include accidental consumption of particles through indiscriminate feeding strategies (e.g. filter-feeders; Besseling et al., 2015; Cole et al., 2013); or active selection due to misidentification of microplastics for food (de Sá et al., 2015; Hall et al., 2015; Neves et al., 2015), based on sensory signals, such as visual or olfactory cues (Hoarau et al., 2014; Savoca et al., 2016). Once ingested, microplastics can cause a reduction in feeding capacity, energy reserves and reproductive output as well as detrimental alterations to intestinal function as shown in a number of low trophic level organisms (Cole et al., 2015; Pedà et al., 2016; Sussarellu et al., 2016; Wright et al., 2013a). Microplastics may also be ingested *indirectly* as a result of trophic transfer, whereby contaminated prey items are consumed by predators (Farrell and Nelson, 2013).

To date, empirical studies have demonstrated that trophic transfer occurs under laboratory conditions for low trophic level organisms, such as crabs (Batel et al., 2016; Farrell and Nelson, 2013; Setälä et al., 2014; Watts et al., 2014), but the extent to which this occurs in the wild and in higher trophic level organisms, is as yet unknown. Studies have recorded microplastic particles within the gastro-intestinal tracts (GIT) of various wild-caught fish species (Lusher et al., 2013; Neves et al., 2015; Rummel et al., 2016), highlighting the potential for transfer to

predators to occur. Marine mammals that employ a raptorial feeding strategy, where prey is captured using the jaws and teeth alone, may be more likely to experience trophic transfer as primary route of microplastic ingestion than through direct intake (Hocking et al., 2017). For example, Lusher et al., (2016) found that 11% of mesopelagic fish investigated contained microplastics and calculated that ~463 million microplastics could be ingested by one striped dolphin (*Stenella coeruleoalba*) through the consumption of contaminated prey. This, however, remains to be demonstrated by empirical research. In seabirds, pellets (regurgitate) from great skuas (*Stercorarius skua*) containing remains of Northern fulmars (*Fulmarus glacialis*) exhibited the highest plastic prevalence, leading the authors to surmise that plastic burden is related to prey type and is therefore a result of trophic transfer (Hammer et al., 2016). Eriksson and Burton (2003) found that scats (faeces) collected from an Antarctic fur seal (*Arctocephalus tropicalis* and/ or *A. gazella*) colony contained plastic particles, ranging from 2 to 5 mm (<0.5 mm were not included in the analysis). The authors suggest that, as the fur seals are unlikely to have ingested plastic of this size directly, the observed microplastic presence could be explained by a 'plastics concentrating stage', whereby a species of fish (*Electrona subaspera)* consume plastic particles from the water column and are in turn predated upon by the fur seals (Eriksson and Burton, 2003). Similar inferences were made for observations recorded for Hooker's sea lions (*Phocarctos hookeri* ; Goldsworthy et al., 1997; McMahon et al., 1999). Another study analysed stomachs, intestines and scats of harbour seals (*Phoca vitulina*) and found the incidence of plastic to be 11%, 1% and 0% respectively (Bravo Rebolledo et al., 2013). The methods used to locate and identify plastic particles, were not appropriate for microplastics and the authors acknowledge the risk of losing 'small and poorly visible' particles and overlooking small particles (0.12 - 0.3 mm) during microscopic sorting. Though deemed unlikely by Eriksson and Burton (2003), the possibility that microplastics found in scat is a result of direct plastic consumption (either accidentally or through naivety) cannot be excluded. For example, twelve of 32 seal species have been documented to ingest marine debris (Kuhn et al., 2015; Ryan et al., 2016) and Hoarau et al. (2014) inferred that small plastic pieces found within marine turtles resulted from fragmentation of larger plastic pieces within the GIT. This indicates that microplastics detected in GITs may have

originally been directly ingested as macro-plastics. Furthermore, external contamination of the scats *in situ*, cannot be discounted. The ethical constraints of subjecting large organisms, such as marine mammals, to laboratory investigations, hinder the resolution of practical issues, such as contamination, experienced by *in natura* studies. Here, we analysed scats from captive seals (residents of a rehabilitation centre) and the wild-caught fish they are fed upon. As a result, the issue of contamination and the likelihood of direct plastic consumption were significantly lessened. The aims of this study were to; a) assess the abundance of microplastics in both scats and fish prey and characterise them by type (fragment or fibre), colour, size and polymer b) evaluate the efficacy of the methods utilised to isolate and identify microplastic particles and c) determine whether microplastic presence can be attributed to trophic transfer.

## Materials and methods

### *Sample collection*

#### *Seal scats and fish*

Seal scats were collected from an outdoor enclosure at the Cornish Seal Sanctuary in Gweek, Cornwall (United Kingdom) containing four resident adult grey seal males. The animals, which are of wild origin, have been residing at the Seal Sanctuary for at least four years and are not exposed to anthropogenic litter items, which may be encountered by wild animals. A plastic enrichment toy, however, is provided. As such, samples were taken from the toy to compare with any particles found in the scats. Two scat samples (approx. 100 ml) were collected per week for 16 weeks, approximately three or four days apart ($n$ = 31). To examine the trophic link and possible transfer of microplastics, fish usually fed to the seals ($n$ = 31) were retained. These were mackerel (mean weight ± SD = 130 ± 22 g; mean length ± SD = 23 ± 2 cm) obtained from a local supplier, caught within the local region (Celtic Sea/English Channel/Western Approaches).

#### *Water samples*

Water for the enclosure pool is pumped from the Helford River via a sediment trap, and though filtered, is a potential source of microplastic contamination. To control for this, water samples (50 ml; $n$ = 31) were collected alongside the scats.

All samples were stored at -20°C prior to further examination.

*Sample preparation: Fish prey items*

*Gastro-intestinal tract and content extraction*

Whole mackerel were thawed at room temperature. An incision was made at the anus, along the ventral side of the fish to the gill covers to expose the internal organs. The gastro-intestinal tract (oesophagus, stomach, pyloric ceca and intestines) was located, removed and rinsed with Milli-Q water (ultrapure and filtered). A syringe was used to flush approximately 50 ml of Milli-Q water through the GIT from the entrance of the oesophagus and the resulting fluid was collected. On a clean metal surface, an incision was made along the length of the GIT. Milli-Q water and a metal spatula were used to extract the GIT content which was collected and contained with the flushed fluid from the previous step. The resulting liquid was then passed through a 40 µm mesh disc using a vacuum pump. The mesh disc was placed inside a Petri dish and dried.

*Sample preparation: Seal scats*

*Sieving*

Scats were thawed at room temperature before being passed through a stack of fractionating sieves (mesh sizes: 2000 µm, 1000 µm, 500 µm and 200 µm) using Milli-Q water and a metal spatula. The material was collected at each level, including 50 ml of liquid contained in the beaker in which the sieves were held, to ensure particles of <200 µm in size were also captured. The collected material was dried at 60˚C until no moisture remained to optimise concentrations of solutions used during enzymatic digestion.

*Enzymatic digestion*

Microplastics present in environmental samples may be masked by biological material. Some methods of removing this material, such as the use of strong oxidizing agents (e.g. acids) can damage or degrade the microplastic particles (Lusher et al., 2017). The use of enzymes, however, is considered a more appropriate method as it does not alter the properties of plastic (Lusher et al., 2017). As such, an enzymatic digestion protocol developed by Lindeque and Smerdon (2003) and adapted by Cole et al., (2014), was further adapted for

application to seal scats. A 3 g subsample (50% ± 15% SD of total scat sample dry weight) of the desiccated material was digested. 15 ml of homogenizing solution (400 ml Tris-HCI buffer, 120 ml ethylenediaminetetraacetic acid (EDTA), 30 ml sodium chloride (NaCl), 100 ml Sodium Dodecyl Sulphate (SDS), 350 ml Milli-Q water) per gram of dried scat was added to a clean (acid washed and rinsed with Milli-Q water) Duran bottle. Samples were physically homogenized by stirring rapidly with a metal spatula for 30 s and incubated at 50˚C for 30 mins. 750 µl of 20 mg $mL^{-1}$ Proteinase-K was added to each gram of dried scat and incubated for up to 24 hours at 50˚C. Following this, 3 ml 5 M sodium perchlorate ($NaClO_4$) was added per gram of dried scat and samples shaken at 20˚C (room temperature) for >30 mins. Samples were again physically homogenized for a longer period of 1 min and then incubated a final time at 60˚C for 30 mins. Each sample was then split across three 40 µm mesh discs using a vacuum pump and subsequently left to dry.

*Microplastic identification*

The physical characteristics of microplastics can facilitate an understanding of their possible sources and reasons for ingestion. As such, material retained on the mesh discs (for fish GITs and seal scats) was visually inspected under a microscope (Olympus SZX16) and any potential plastic particles were classified (type - fragment or fibre; colour; size and description), photographed (using a microscope mounted Canon EOS 550D DSLR camera) and retained separately for further analysis using Fourier-Transform Infrared (FTIR) spectroscopy (Agilent Cary 630 FTIR spectrometer; Agilent FTIR Spectral Library – Poly 8). Microplastic colour was determined by eye.

When interpreting FTIR output, only readings with confidence levels of 70% or greater (Lusher et al., 2013) and those considered to have reliable spectra matches (after visual inspection) were accepted. Only these particles were included for further analysis. All confirmed synthetic polymer particles were included in our results.

*Contamination and microplastic loss prevention*

Contamination of samples by microplastics present on equipment and within the atmosphere risks producing inaccurate results and should therefore be

minimised. In addition, their small size means that microplastics present within the samples may be lost during processing. A number of measures, listed below, were implemented to limit these risks and control for any contamination that did occur.

*Sample collection*

Sample collection pots were thoroughly rinsed with Milli-Q water in a clean environment. Scat collection was carried out using a metal scraping instrument and sample pot caps were removed for as limited time as possible.

*Sample preparation*

Throughout the sample preparation process, a cotton lab coat and gloves were worn. All work surfaces were wiped down with 70% ethanol prior to any work commencing and all equipment was thoroughly rinsed with Milli-Q water.

*Sieving*

Work was carried out inside a positive pressure laminar flow hood. Prior to use and between scats, the sieves were scrubbed using a natural fibre brush and veterinary detergent and then rinsed thoroughly with Milli-Q water. Damp filter paper in a Petri dish was used to control for any air-borne contamination inside the flow hood where the work was carried out. In addition, a procedural blank (20 ml Milli-Q water) was run through the sieves and filtered using a mesh disc to control for any contamination at this stage of processing. Both the mesh disc and filter paper were inspected under a microscope for any particles at the beginning and end of this step respectively.

*Enzymatic digestion*

Prior to any work, all equipment was rinsed with Milli-Q and all pipettes and syringes were flushed with Milli-Q. Lids were removed from Duran bottles for as limited time as possible. Scats were weighed in an enclosed balance. After homogenizing, the metal spatula was rinsed with homogenizing solution to avoid loss of particles from samples. The vacuum pumping process was carried out inside the laminar flow hood. Prior to vacuum pumping all mesh discs were visually inspected for contamination under a microscope and any particles

removed. A procedural blank was run through the vacuum pump and the mesh disc inspected before samples were filtered. If contamination was found, the vacuum pump and mesh disc were cleaned until no particles were detected.

## Results

*Microplastic presence in fish prey*

Of the individual fish examined (*n* = 31), 10 (32%) contained 18 confirmed microplastic particles (Table 1).

**Table 1.** Physical characteristics and Fourier-Transform Infrared (FTIR) spectroscopy output for plastic particles found in fish and seal scats.

| Sample | Type | Colour | Size (µm) | Polymer | FTIR | Spectra |
|---|---|---|---|---|---|---|
| Fish | Fibre | Blue | 5000 x 30 | NBR | 0.808 | Reliable |
| | Fibre | Black | 4200 x 50 | Polyacrylamide | 0.888 | Reliable |
| | Fibre | Red | 2000 x 30 | Neoprene | 0.845 | Reliable |
| | Fibre | Orange | 2500 x 30 | Polyethylene | 0.893 | Reliable |
| | Fragment | Red | 700 x 200 | Aramid woven fabric | 0.702 | Reliable |
| | Fragment | Red | 300 x 100 | Polyethylene | 0.849 | Reliable |
| | Fibre | Red | 2000 x 100 | Polyethylene | 0.834 | Reliable |
| | Fragment | Orange | 100 x 100 | EPDM | 0.865 | Reliable |
| | Fragment | Green | 100 x 100 | Polyethylene | 0.823 | Reliable |
| | Fibre | Red | 700 x 50 | Ethylene Propylene | 0.832 | Reliable |
| | Fibre | Black | 4000 x 30 | Poly (butylene terephthalate) | 0.814 | Reliable |
| | Fibre | Blue | 50 x 50 | Neoprene | 0.874 | Reliable |
| | Fibre | Black | 1200 x 30 | Polyethylene | 0.851 | Reliable |
| | Fibre | Black | 2500 x 30 | Ethylene propylene | 0.768 | Reliable |
| | Fibre | Blue | 6000 x 30 | Ethylene Propylene | 0.881 | Reliable |
| | Fibre | Blue | 3300 x 50 | Ethylene propylene | 0.838 | Reliable |
| | Fibre | Blue | 1800 x 50 | Ethylene Propylene | 0.859 | Reliable |
| | Fragment | Green | 200 x 150 | Polypropylene | 0.875 | Reliable |
| Seal scats | Fragment | Red | 500 x 500 | Polypropylene | 0.81 | Reliable |
| | Fragment | Clear | 2600 x 400 | Polypropylene | 0.81 | Reliable |
| | Fragment | Clear | 800 x 600 | Polypropylene | 0.93 | Reliable |
| | Fibre | Black | 600 x 50 | Ethylene propylene | 0.88 | Reliable |
| | Fragment | Red | 1000 x 400 | Polyethylene | 0.91 | Reliable |
| | Fibre | Black | 1200 x 50 | Ethylene propylene | 0.88 | Reliable |
| | Fibre | Black | 2100 x 10 | Ethylene propylene | 0.89 | Reliable |
| | Fibre | Black | 1300 x 10 | Ethylene propylene | 0.92 | Reliable |
| | Fragment | Red | 1200 x 900 | Polyethylene | 0.77 | Reliable |
| | Fibre | Black | 600 x 50 | Ethylene propylene | 0.95 | Reliable |
| | Fragment | Black | 2500 x 100 | Polyacrylamide | 0.84 | Reliable |
| | Fragment | Red | 500 x 600 | Polyurethane | 0.83 | Reliable |
| | Fragment | Clear | 5500 x 400 | Polypropylene | 0.71 | Reliable |
| | Fragment | Blue | 400 x 300 | Ethylene propylene | 0.84 | Reliable |
| | Fragment | Orange | 1800 x 1200 | Ethylene propylene | 0.85 | Reliable |
| | Fragment | Black | 700 x 100 | Polyaramid Kevlar | 0.77 | Reliable |
| | Fragment | Orange | 3500 x 2300 | EPDM | 0.87 | Reliable |
| | Fragment | Red | 600 x 300 | Polypropylene | 0.89 | Reliable |
| | Fibre | Clear | 3500 x 100 | Polyethylene | 0.84 | Reliable |
| | Fibre | Blue | 600 x 500 | Styrene butadiene rubber | 0.83 | Reliable |
| | Fragment | Clear | 2300 x 1500 | Neoprene | 0.86 | Reliable |
| | Fragment | Blue | 1000 x 800 | Styrene butadiene rubber | 0.88 | Reliable |
| | Fibre | Red | 2300 x 50 | Polypropylene | 0.82 | Reliable |
| | Fragment | Clear | 20 x 800 | NBR | 0.78 | Reliable |
| | Fragment | Orange | 1100 x 700 | Polyacrylamide | 0.86 | Reliable |
| | Fragment | Blue | 500 x 400 | Polypropylene | 0.86 | Reliable |

The number per fish ranged from 0 – 4 (mean ± SD = 0.58 ± 1.05 particles; Fig. 1a). The majority were fibres (*n* = 13; 72%) and the remaining 28% comprised of fragments (*n* = 5). The most prevalent colours were red and blue (both 28%), black (22%), orange and green (both 11%; Fig. 1c).

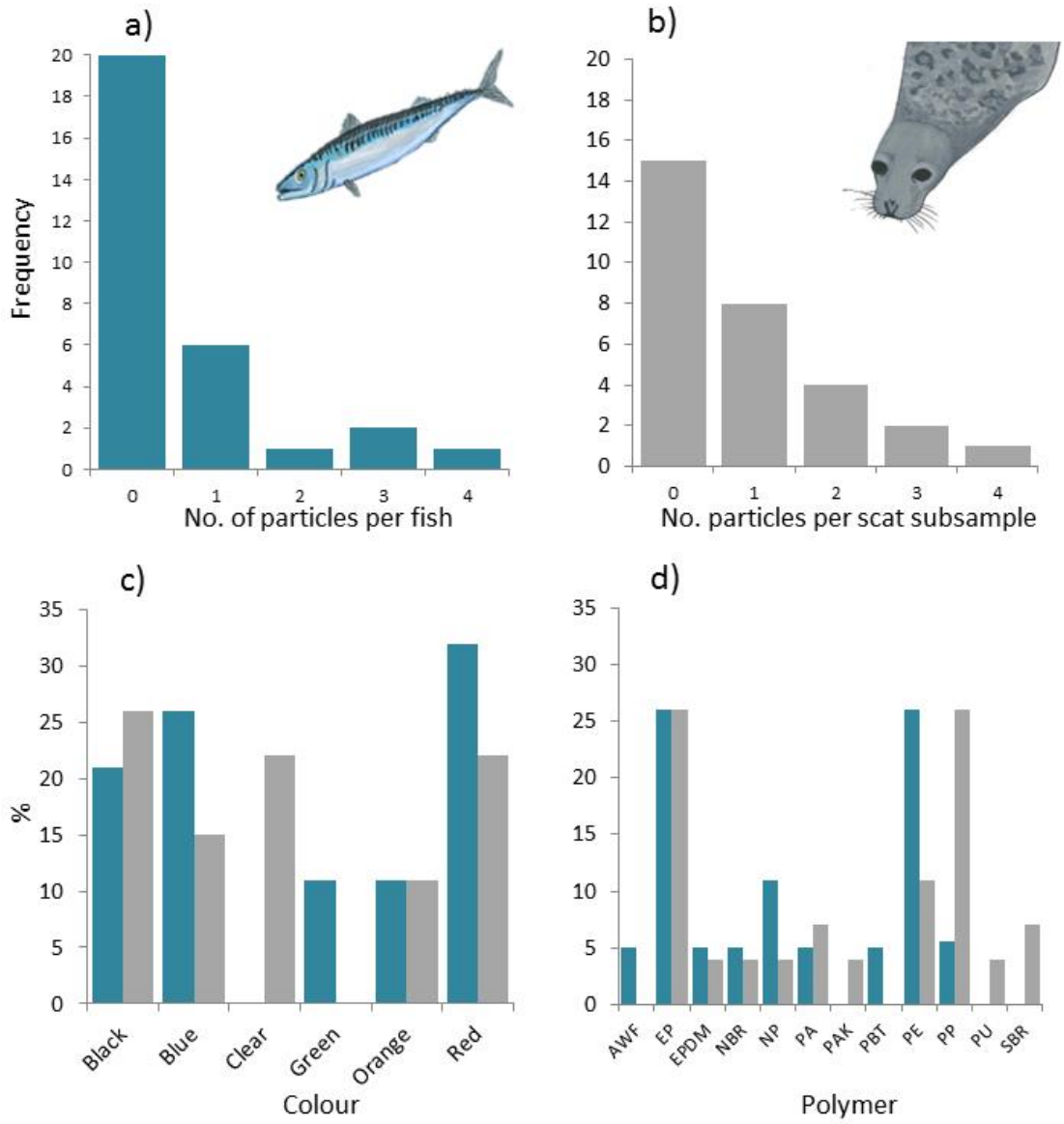


**Fig. 1. a)** Frequency histogram showing number of plastic particles per fish; **b)** Frequency histogram showing number of particles per scat subsample; **c)** Barplot showing percentage of particles for each colour in fish (blue) and scats (grey); **d)** Barplot showing percentage of particles per polymer type for fish (blue) and scats (grey; AWF = aramid woven fabric; EP = ethylene propylene; EPDM = ethylene propylene diene monomer (M-class) rubber; NBR = nitrile butadiene rubber; NP = neoprene; PA = polyacrylamide; PAK = polyaramid Kevlar; PBT = poly (butylene terephthalate); PE = polyethylene; PP = polypropylene; PU = polyurethane; SBR = styrene butadiene rubber).

Fibres ranged from 0.5 to 6.0 mm in length. The largest fragment found was 0.7 x 0.2 mm and the smallest 0.1 x 0.1 mm in diameter. The mean particle length was 2.0 mm (± SD = 1.8 mm). The most prevalent polymer types as confirmed by FTIR were ethylene propylene and polyethylene (both 28%) followed by neoprene (11%), polypropylene, ethylene propylene diene monomer (EPDM), nitrile butadiene rubber (NBR), aramid woven fabric, poly (butylene terephthalate), polyacrylamide (all 6%; Fig. 1d). See Fig. 2a for photographic examples of microplastics found in fish.

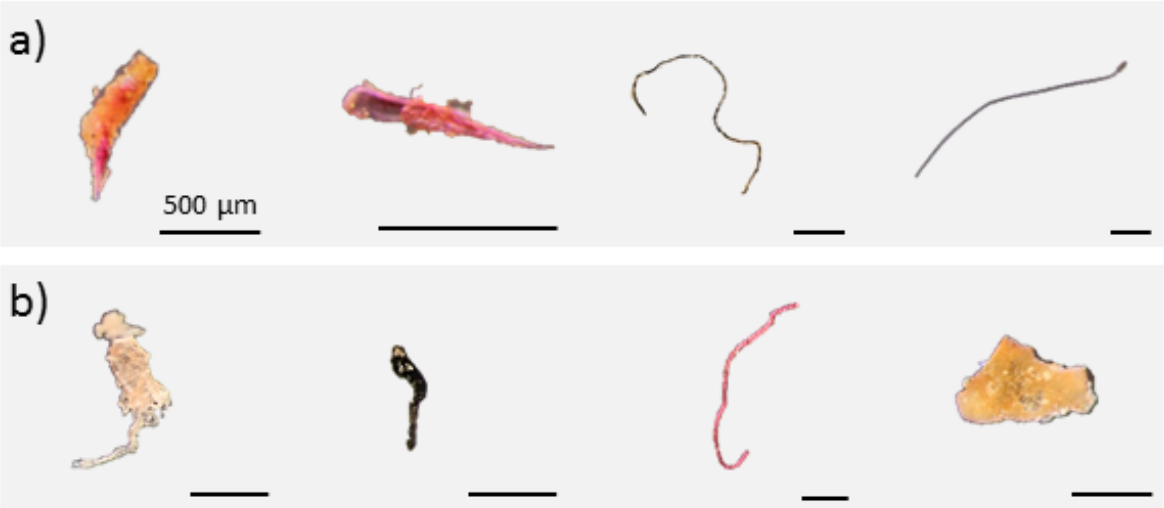


**Fig. 2** Photographic examples of particles found in **a)** fish (from l-r: aramid woven fabric, polyethylene, ethylene propylene, polyacrylamide) and **b)** scat subsamples (from l-r: polyethylene, polyaramid Kevlar®, polypropylene, polyacrylamide). Scale bars represent 500 µm.

*Microplastic presence in scats*

Of the 31 scat subsamples analysed, 15 (48%) contained a total of 26 confirmed microplastic particles (Table 1). The number of particles per scat ranged from 0 – 4 (mean ± SD = 0.87 ± 1.09 particles; Fig. 1b). Of these, 18 were fragments (69%) and eight were fibres (31%). Black particles were most commonly found (27%) followed by clear (transparent) and red (both 23%), blue (15%) and orange (12%; Fig. 1c). Particle size varied with fragments ranging from 0.4 x 0.3 mm to 5.5 x 0.4 mm. Fibres ranged from 0.6 to 3.5 mm in length. The mean particle length was 1.5 mm (± SD = 1.2 mm). The most common polymer types identified by FTIR were ethylene propylene and polypropylene (both 27%) followed by polyethylene (12%), polyacrylamide and styrene butadiene rubber (both 8%),

neoprene, EPDM, NBR, polyaramid Kevlar, polyurethane (all 4%; Fig. 1d). These results are from scat sub-sample representing ~50% of total dry weight. See Fig. 2b for photographic examples of microplastics found in scats.

*Contamination levels*

*Water samples and enrichment toy*

Black ethylene propylene fibres (*n* = 4) were detected in water samples taken from the enclosure pool but as these were also found in the fish GITs, those detected in the scats were included within the results. It is likely that the seals defecated in the pool and so introduced the particles themselves. No particles matching the enrichment toy were detected.

*Sample preparation*

No evidence of contamination was found in any of the procedural controls or blanks. Blue polypropylene fragments (*n* = 5) matching FTIR output for the bottle lids used during sample preparation were found in two of the samples. These were excluded from the results as these were considered to be a possible result of contamination. Aluminium foil lids were used for the remaining samples to avoid any further possibility of contamination.

## Discussion

This study is the first to investigate and demonstrate empirical evidence for the trophic transfer of microplastics from fish to a marine top predator. Studies on microplastics and pinnipeds are scarce (Bravo Rebolledo et al., 2013), making it challenging to draw comparisons with our results. A wild study found the number of particles per scat ranged from 0 - 4 and the majority of those containing microplastics had one particle (Eriksson and Burton, 2003). It is not clear whether the whole scat or a subsample was examined, or what methods were employed to do so. In this study black, clear and red were the most frequently found colour particles in scats which differs from Eriksson and Burton (2003) where white, brown, blue, green and yellow were most common. Additionally, the mean particle length was 4.1 mm which differs from our result (1.5 mm; Eriksson and Burton, 2003). It is possible that methodological techniques employed in our study allowed for smaller particles to be detected. Though not discussed explicitly, it

seems that all particles found were fragments, which is similar to the results of our study, though some fibres were identified.

Ingestion rates of microplastics by fish prey could not be accurately assessed in this study because samples were obtained from the fishing industry and not collected using the necessary sampling protocols. This is important because some species of fish are known to regurgitate stomach contents during capture as a result of handling stress which may result in the loss of microplastics and so bias the results of any analysis (Bromley, 1994; Lusher et al., 2017). Conversely, during capture, fish may ingest microplastics that accumulate in the net, or originate from the net itself (Lusher et al., 2013). Nevertheless, Neves (2015) found that 31% Atlantic mackerel sampled had ingested microplastics, with a mean of 0.46 (±0.78) microplastics per individual. This corresponds with the results of this study, whereby 32% of mackerel contained microplastics with mean of 0.58 particles per fish. Our finding that fibres were more commonly detected (72%) than fragments corresponds with findings from other research on environmental microplastic concentrations (Claessens et al., 2011; Lusher et al., 2013; Neves et al., 2015; Wright et al., 2013b) and two studies investigating fish found approximately 66% and 68% of microplastics were fibres (Lusher et al., 2013; Neves et al., 2015). One study reported particles of various colours with the black being the most common at 45% (Lusher et al., 2013). We found black to be the third most common colour (22%) after red and blue. Neves (2015) found the size of particles generally ranged from 0.217 to 4.81 mm (mean 2.11 ±1.67 mm) and Lusher (2013) reported a larger range of 0.13 - 14.3 mm the most common size class to be 1–2 mm. The mean particle length detected in fish in our study was 2 mm.

In total, 12 polymer types were detected in the fish and scats analysed in this study. The most common for both was ethylene propylene, indicating a clear link between the seals and the fish they consumed. The particles detected in scats by Eriksson and Burton (2003) comprised five major polymer groups; polyethylene (93%), polypropylene (4%), poly(1-Cl-1-butenylene) polychloroprene (2%), melamine-urea (phenol) (formaldehyde) resin (0.5%) and cellulose (0.5%). The polymer types detected in the scats of our study were more varied (10 polymer types), which may be as a result of diversity within the marine environment. The animals investigated by Eriksson and Burton (2003) were

located on Macquarie Island, a remote island in the southwest Pacific Ocean. As such, they are likely exposed to different microplastic inputs from those in our study, which are fed on fish from the north-east Atlantic, caught near the British coast. The two most common polymers detected in fish by Neves (2015), polypropylene and polyethylene, were also commonly detected in the scat and fish analysed in this study.

Our findings indicate some disparities between the type, colour and size of microplastics detected in fish compared with those found in scats. For example, the majority of particles detected in scats were fragments while the reverse is true for the fish with fibres being most common. Though black, red and blue particles featured prominently in both fish and scats, and they contained the same proportion of orange particles, the latter also contained a high proportion of clear particles which were not detected in fish. A range of sizes of fragments and fibres were detected. These variations may be due to several reasons;

*Diversity within the system:* The fish examined for microplastics may not have been caught concurrently with those fed to (and excreted by) the seals. As a result of the considerable diversity in microplastic abundance, type (fragment/ fibre), size, colour and polymer observed not only among fish (individuals, populations and species (Lusher et al., 2013; Neves et al., 2015; Rummel et al., 2016) but within the marine environment generally (Amélineau et al., 2016; Cózar et al., 2015; Woodall et al., 2014), we would not expect to see a complete match between the particles found in the scats and the fish.

*Methodological constraints:* The differing methods of microplastic extraction and isolation from fish GITs and scat may have contributed to some of the observed variation. For example, though efforts were made to minimise microplastic loss, it is possible that the protracted processing involved in enzymatic digestion of the scats, increased the risk of losing some particles. In addition, microplastic detection relies on human ability so it is likely that particles that are ‘natural’ in colour (i.e. brown, beige) are under reported in some cases. The colour of background substrate may influence which colours are more likely to be detected. For example, clear/ transparent particles are less obvious in fish than scat because the substrate is translucent. The relatively small sample sizes are also likely to have contributed to some of the observed variation.

*Biological implications:* One study found more plastic in the stomachs of harbour seals (*Phoca vitulina*) than elsewhere in the GIT or scat (Bravo Rebolledo et al., 2013). This suggests that the stomach may act as a trap for non-food items, such as microplastics. To investigate this further, it would be necessary to examine the GITs of dead animals, preferably those known to have died as a result of physical trauma, such as by-catch, whereby normal feeding behaviour prior to death can be assumed.

It has been suggested that atmospheric microplastics may be a source of particles found in the gut content/ faeces of marine mammals (Lusher et al., 2017). Though this is possible in some cases, it is unlikely in this study for a number of reasons. Firstly, most atmospheric microplastics are fibres (Dris et al., 2016) and the majority of particles found in the scats were fragments. Secondly, the animals investigated in this study reside in a rural area, with very low levels of air pollution (www.uk-air.defra.gov.uk/air-pollution; last accessed 16 October 2017). Lastly, the strong correlation between polymer type in both fish and seal scats indicates that the microplastics found in scats were a consequence of ingestion as opposed to inhalation or contamination. It is unknown to what extent wild animals are exposed to atmospheric microplastics but examination of the lungs and airways of stranded animals could be a worthy aspect for future research efforts, alongside the monitoring of atmospheric microplastic levels at sea.

The methods of microplastic extraction and contamination control used in this study were effective for determining the presence and characteristics of microplastic particles in fish and scat. In addition, the use of captive seals significantly reduced the possibility of direct plastic consumption. As such, we attribute the presence of microplastic particles in seal scats to the occurrence of trophic transfer from prey to a marine top predator. Whether these particles were directly consumed by the fish or underwent trophic transfer from ingestion of contaminated zooplankton is not known. Mackerel in the north east Atlantic, though opportunistic, feed primarily on calanoid copepods (Bachiller et al., 2016), which are approximately 2 mm in length (Lindeque et al., 2006). Zooplankton can consume microplastic particles of 0.4−30.6 μm in size (Cole et al., 2013) but all microplastics found in the fish were considerably larger than this (>100 μm) with a mean size of 2 mm. This indicates that microplastics found within the mackerel

were most likely consumed directly from the water column, possibly because they were mistaken for prey items. Similarly, Amberstripe scad (*Decapterus muroadsi*) have been shown to readily ingest microplastics resembling their copepod prey in colour and size (Ory et al., 2017). The authors surmise that planktivorous fish are more likely to consume microplastics directly because of their feeding ecology as visual predators (Ory et al., 2017). Further investigation is needed to understand selectivity and its impacts on trophic transfer.

The occurrence of microplastic trophic transfer may have a number of impacts for top predators;

*Physiological implications:* Microplastic ingestion has been shown to cause a number of detrimental physiological impacts resulting in a reduction in feeding capacity, energy reserves and reproductive output for smaller low-trophic level organisms (Cole et al., 2013; Sussarellu et al., 2016; Wright et al., 2013a). It is not yet known whether this occurs in larger animals, such as marine mammals. Furthermore, very little information exists regarding the retention time of microplastics in marine mammal GITs. A study investigating the prey passage time of grey seals found that the majority of fish otoliths (ear bones) could be recovered from scats ~88 hours after consumption (Grellier and Hammond, 2006; Lusher et al., 2016). The feeding trial also found that all polystyrene beads (3 mm) were recovered after 6 days. This suggests that, although they may take longer, microplastics are egested alongside indigestible dietary items (Lusher et al., 2016). It is not known, however, what effect this partial retention has on digestive processes and whether fibres behave differently within the digestive tract to the beads used by Grellier and Hammond (2006).

*Prey availability:* The known impacts for low trophic level organisms may have secondary implications for predators in the form of reduced food availability, i.e. Increased mortality of prey species as a result of microplastic ingestion. Further research is needed to assess whether this is the case.

*Microplastics and chemical contaminants:* Biomagnification and bioaccumulation of chemical contaminants, such as polychlorinated biphenyls (PCBs) and organochlorine pesticides (OCPs), are known to occur at higher trophic levels, particularly affecting marine top predators (Jepson et al., 2016; Tsygankov et al., 2015). Whether a similar mechanism occurs for microplastics is unknown. For example, does the abundance of microplastic particles increase

through and up marine food webs, and with the age of the animal? Further research is needed to investigate whether animals at higher trophic levels experience higher plastic loads than those at lower levels and whether older animals experience higher abundances than younger ones of the same species/ population. In addition, microplastics may act as a vector for transporting chemicals, both trophically (Teuten et al., 2007) and spatially. For example, population declines in some marine mammal species have been linked to elevated burdens of OCs as a result of their presence within the marine environment (Murphy et al., 2015). The large surface area to volume ratio of microplastics can lead to the adsorption and concentration of such hydrophobic toxicants (Teuten et al., 2007). If consumed, they may desorb into biological tissues, potentially leading to detrimental endocrine and/ or immune system effects with implications for reproductive success (Jepson et al., 2016; Murphy et al., 2015; Teuten et al., 2009). The ingestion of microplastics may represent an additional pathway by which these chemicals enter marine mammals, aside from the usual dietary input.

*Human health:* Our finding that microplastics can be transferred from fish to top predators has implications for human health. For instance, seafood that is consumed whole (i.e. including the GIT), such as shellfish, has been found to contain microplastics (Murray and Cowie, 2011; Rochman et al., 2015; Van Cauwenberghe and Janssen, 2014). Further work is required to better understand the extent of exposure to and impacts of microplastic ingestion on humans.

## Conclusion

We present empirical evidence that microplastic particles can be transferred across trophic levels, from fish to a marine mammal top predator. Our findings suggest that trophic transfer represents an indirect, yet potentially major, pathway of microplastic ingestion for any species whose feeding ecology involves the consumption of whole prey.

# Chapter 3: Microplastics in marine mammals stranded around the British coast: ubiquitous but transitory?

This chapter is a reformatted copy of my publication: **Nelms SE,** Barnett J, Brownlow A, Davison NJ, Deaville R, Galloway TS, Lindeque PK, Santillo D, Godley BJ (2019) Microplastics in marine mammals stranded around the British coast: ubiquitous but transitory? *Scientific Reports*. 9 (1) 1075. I conducted all of the sample processing and analysis and was lead author on this work; JB, AB, ND and RD provided the samples; DS provided training and access to essential equipment; BJG guided the development of the project; all authors provided comments and edits to help shape the final manuscript. **Impact: Citations – 2; Altmetric score - 1217**


## Abstract

Plastic pollution represents a pervasive and increasing threat to marine ecosystems worldwide and there is a need to better understand the extent to which microplastics (<5 mm) are ingested by high trophic-level taxa, such as marine mammals. Here, we perform a comprehensive assessment by examining whole digestive tracts of 50 individuals from 10 species whilst operating strict contamination controls. Microplastics were ubiquitous with particles detected in every animal examined. The relatively low number per animal (mean = 5.5) suggests these particles are transitory. Stomachs, however, were found to contain a greater number than intestines, indicating a potential site of temporary retention. The majority of particles were fibres (84%) while the remaining 16% was fragments. Particles were mainly blue and black (42.5% and 26.4%) in colour and Nylon was the most prevalent (60%) polymer type. A possible relationship was found between the cause of death category and microplastic abundance, indicating that animals that died due to infectious diseases had a slightly higher number of particles than those that died of trauma and other drivers of mortality. It is not possible, however, to draw any firm conclusions on the potential biological significance of this observation and further research is required to better understand the potential chronic effects of microplastic exposure on animal health, particularly as marine mammals are widely considered important sentinels for the implications of pollution for the marine environment.

## Introduction

Marine mammals, such as whales, dolphins and seals, are often considered important indicators of marine ecosystem health, particularly in relation to pollution (Bossart, 2011; Mössner and Ballschmiter, 1997). The high-trophic level status and long life-span of some species leaves them susceptible to bioaccumulation and biomagnification of aquatic chemical contaminants, which have been shown to cause population-level effects (Jepson et al., 2016; Murphy et al., 2015; Pierce et al., 2008). As a result of this and other anthropogenic stressors, many species of this taxonomic group are of conservation concern (Parsons et al., 2015). Ingestion of anthropogenic litter by marine mammals has been documented in a number of species (n = 123; Kuhn et al., 2015), yet the number of studies (which use appropriate methods of extraction and contamination control) investigating the physical presence of microplastics (<5 mm in size) in the digestive tracts of cetaceans is extremely low (*n* = 4; totalling 57 animals of 8 species from Ireland, the Netherlands and Spain (Besseling et al., 2015; Hernandez-Gonzalez et al., 2018; Lusher et al., 2015, 2018); polymer information has been presented for two animals only (Besseling et al., 2015; Lusher et al., 2015) and there are no studies whereby the digestive tracts of wild pinnipeds have been examined.

Microplastics in the marine environment originate from a variety of sources, including fragmentation of larger macro-plastic debris, pre-production pellets (nurdles) spilled during transportation and fabrication, outflow of wastewater containing microbeads from cosmetics and fibres from the washing of synthetic textiles, as well as road-run-off containing fragments of vehicle tyres and marking paint (Andrady, 2011; Barnes et al., 2009; Boucher and Friot, 2017; Browne et al., 2011; Cole et al., 2011; UNEP, 2009). Their small size makes them highly bioavailable to ingestion by a wide variety of marine biota from zooplankton, such as copepods, other invertebrates (including shellfish), both juvenile and adult fish, seabirds and marine megafauna (Amélineau et al., 2016; Besseling et al., 2015; Cole et al., 2013; Desforges et al., 2015; Farrell and Nelson, 2013; Lusher et al., 2013, 2015; Steer et al., 2017; Watts et al., 2014).

Microplastics may be ingested directly through accidental consumption, for example as a result of indiscriminate feeding strategies, such as filter-feeding (e.g. mysticete whales; Besseling et al., 2015) or indirectly as a result of trophic

transfer, whereby predators consume prey items contaminated with microplastics (Farrell and Nelson, 2013), for example, during raptorial feeding (e.g. most seals and dolphins; Hocking et al., 2017). Though little is known about the extent to which trophic transfer occurs in the wild, the presence of microplastics in scats of captive grey seals (*Halichoerus grypus*) has been attributed to trophic transfer from the wild-caught mackerel (*Scomber scombrus*) they were fed upon (Nelms et al., 2018).

Due to the difficulties of investigating the occurrence and effects of microplastics in the field, many studies are limited to low-trophic level organisms in a laboratory setting. In such cases, ingestion of microplastics has been shown to cause a reduction in feeding and energy reserves as well as impacts on reproductive output and damage to brain and intestinal function in invertebrates and fish (Cole et al., 2013; Lei et al., 2018; Mattsson et al., 2017; Sussarellu et al., 2016; Wright et al., 2013a). In addition, the hydrophobic properties of plastics means that organic chemical contaminants present within seawater, such as polychlorinated biphenyls (PCBs), have a tendency to adsorb to their surface (Teuten et al., 2009). These, and other chemicals added during production, such as plasticisers, can desorb into biological tissue if ingested and cause detrimental effects for organism health, such as oxidative and hepatic stress (Browne et al., 2013; Rochman et al., 2013).

In this study we sought to investigate the extent of microplastic ingestion in wild marine mammals by examining the digestive tracts of a large sample ($n$ = 50) of individuals from 10 species (cetacean $n$ = 43, 8 species; pinniped $n$ = 7, 2 species) that stranded around the coast of Britain. We sought to not only determine the general abundance of microplastics ingested and polymers involved, but also to determine whether microplastics are egested or retained within the digestive tract.

## Results

### *Microplastic abundance*

Every animal was found to contain at least one synthetic particle (See Fig. 1a for photographic examples). In total, 273 particles were detected and 261 of these were less than 5mm in size (mean ± SD = 5.5 ± 2.7 particles per animal; range 1-12 particles). Only one animal was found to contain macroplastics; green

netting in the forestomach of a juvenile short-beaked common dolphin (*Delphinus delphis*).

The majority of particles were fibres (84%; *n* = 229) while the remaining 16% (*n* = 44) was fragments. Particles were mainly blue and black (42.5% and 26.4%, respectively) followed by clear (12.8%), red (11%), green (2.9%), orange and yellow (both 1.5%) and white and multi-coloured (both 0.7%; Fig. 1b.) Fibres ranged in size from 2 cm in length to 0.1 mm (100 µm) with a mean length of 2 mm (± 2.3 mm; Fig.1c). Fragments were between 4 x 2 mm and 100 x 100 µm in size (mean length = 0.9 mm ± 1.1). All (100%; *n* = 50; one per animal) of the particles successfully tested using Fourier Transform Infrared (FTIR) spectroscopy were synthetic, with Nylon the most prevalent (60%; *n* = 30) followed by polyethylene terephthalate (PET) and polyester (all 10%; *n* = 5), phenoxy resin, polyethylene, polypropylene and rayon (all 4%; *n* = 2), polyamide resin and LDPE (both 2%; *n* = 1; Fig. 1d).

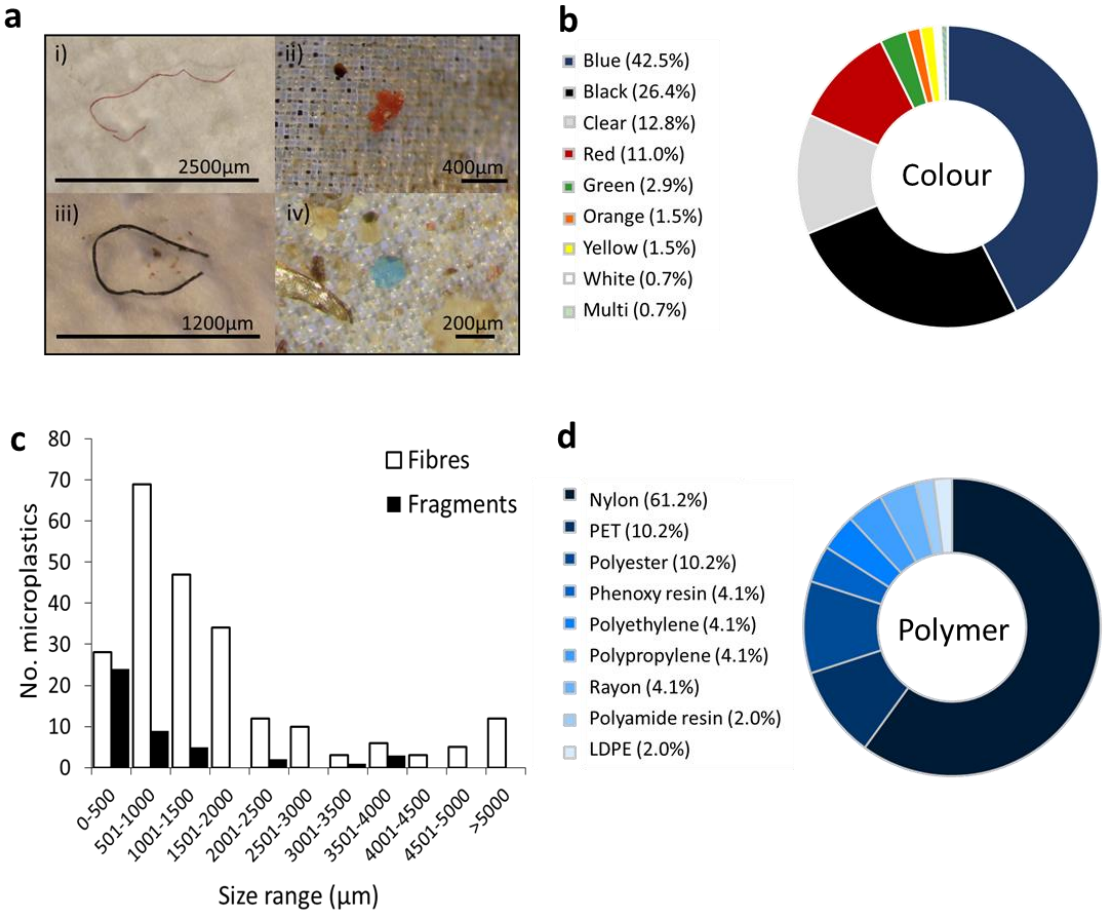


**Fig. 1 a)** Photographic examples of microplastics found in marine mammal digestive tracts (i) Nylon; ii) Polyethylene; iii) Polyethylene terephthalate (PET); iv) Phenoxy resin **b)** proportion of particle colours found in all animals (blue; 42.5%, black; 26.4%, clear; 12.8%, red; 11%, green; 2.9%, orange and yellow;

both 1.5%, white and multi-coloured; both 0.7%) **c)** size ranges (0 - > 5000 µm) of plastic particles found in all animals. Note: a small proportion of fibres were larger than 5mm but were not macroscopically visible and are included here. **d)** the proportion of polymer types found (Nylon; 60%, polyethylene terephthalate (PET) and polyester; both 10%, phenoxy resin, polyethylene, polypropylene and rayon; all 4%, polyamide resin and LDPE; both 2%).

*Factors affecting microplastic abundance*

When we investigated factors that may affect microplastic burden (taxon, age-class, sex, length, cause of death), model simplification indicated that cause of death was the only significant predictor of microplastic abundance ($p$ = 0.01; Supplemental Table S1 and S2) and the mean number of microplastics was significantly different among the three cause of death categories (one-way ANOVA, $F_{2, 47} = 4.31$, $p < 0.05$; Fig. 2). Animals presenting infectious diseases contained slightly higher mean (± SD) microplastics abundances (7.0 ± 2.7), followed by trauma (4.7 ± 2.1) and other (4.6 ± 3.2). This was also the case when we only analysed species (harbour porpoise and common dolphin) with sample size greater than 16 individuals. See Supplemental Table S3 and S4 for further detail.

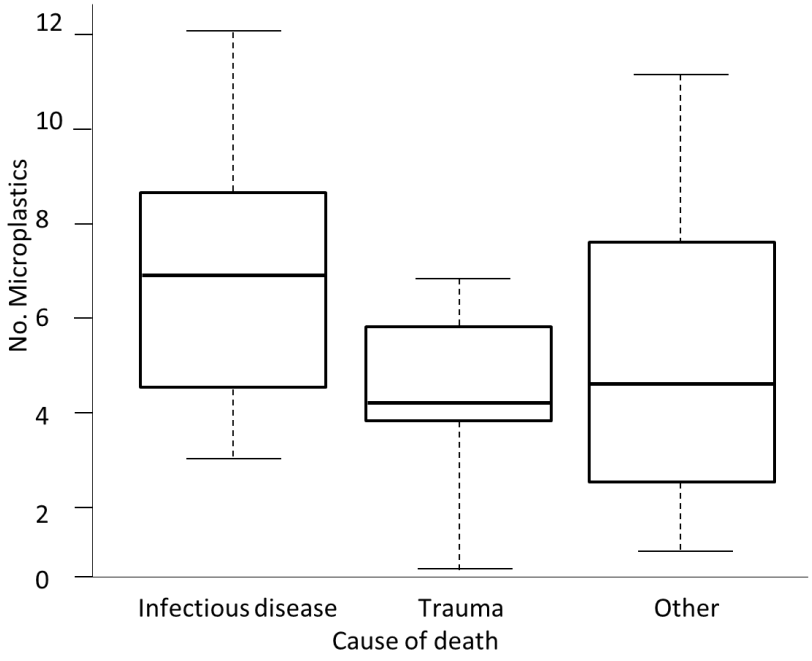


**Fig. 2** Box plot showing the number of microplastics in relation to cause of death category (infectious disease (7.0 ± 2.7), trauma (4.7 ± 2.1), other (4.6 ± 3.2)). The

horizontal black lines represent median values, the boxes depict the first and third quartiles and the whiskers illustrate the minimum and maximum values.

*Distribution of microplastics within the digestive tract*

Of the GIT sections, stomach(s) showed a significantly higher abundance of microplastics (mean ± SD = 3.8 particles ±2.5) than intestines (1.7 ±1.4; one-way ANOVA, $F_{1, 98} = 27.69$, $p < 0.001$; Fig. 3.). There was no significant difference among the compartments of cetacean stomachs (fore, fundic and pyloric; ANOVA, $F_{2, 77} = 0.6472$, $p = 0.5$).

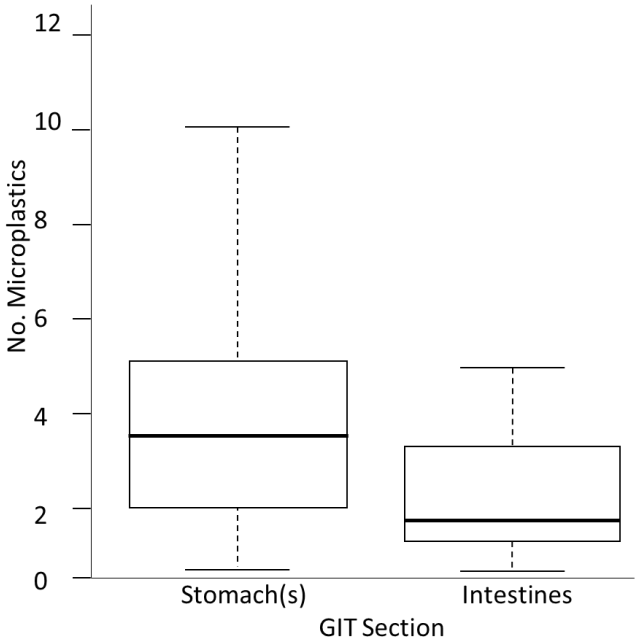


**Fig. 3** Box plot showing the number of microplastics detected in the gastro-intestinal tract (GIT) sections stomach(s) and intestines (mean ± SD = 3.8 particles ±2.5 and 1.7 ±1.4 respectively). The horizontal black lines represent median values, the boxes depict the first and third quartiles and the whiskers illustrate the minimum and maximum values.

*Contamination*

No particles matching the contamination controls were found in any of the samples and all procedural blanks were clear, demonstrating that the measures implemented to minimise contamination were 100% effective.

## Discussion

Our study is the first to assess the presence of microplastics in the digestive tracts of multiple individuals from a range of both cetacean and pinniped species. At least one microplastic, which was confirmed using FTIR, was discovered in every animal with an average of 5.5 particles per animal. There are few studies available for comparison but two studies examined the stomach contents of 35 common dolphins and digestive tracts of 21 cetaceans (of various species) and found a total of 411 and 598 small debris items respectively (Hernandez-Gonzalez et al., 2018; Lusher et al., 2018). Neither study, however, presented FTIR data confirming polymer type. Sixteen confirmed microplastics were found in an unknown volume of gut content from a humpback whale (*Megaptera novaeangliae;* Besseling et al., 2015).

All animals examined in the current study were raptorial feeders, using their jaws and teeth alone to catch prey (Hocking et al., 2017). As raptorial feeders expel seawater through their teeth so as not to ingest it, we presume they are less likely to consume microplastics directly and more likely to indirectly consume them through trophic transfer from contaminated prey (Nelms et al., 2018). However, given that approximately 11-30% of fish contain microplastics (Lusher et al., 2013; Neves et al., 2015) a greater number could perhaps be expected in the digestive tracts of marine mammals than demonstrated here. There are at least three possible explanations for the observed low abundances of microplastics. Firstly, microplastics are egested along with other dietary waste, such as fish bones, otoliths and squid beaks, as shown by their presence in seal scats and the intestines of both cetaceans and seals (Eriksson and Burton, 2003; Lusher et al., 2015; Nelms et al., 2018). A feeding trial examining the passage time of prey in grey seals found the majority of otoliths were passed within four days of consumption and all polystyrene balls (3 mm) fed to the animals were recovered within six days, demonstrating that, although microplastics have a slower passage time, they are egested in the faeces (Grellier and Hammond, 2006; Lusher et al., 2016) . Our finding of higher microplastic abundances in the stomach(s) than intestines, may explain this delay in passage time - the stomach(s) acts as an entrapment site within the digestive tract, partially retaining the microplastics. In addition to egestion, cetaceans, particularly odontocetes (toothed whales) are known to regurgitate foreign objects from the forestomach

(Levine et al., 2014; Mintzer et al., 2008), although very little information exists on the regurgitation rates of wild odontocetes (Mintzer et al., 2008). Furthermore, a study on low trophic level organisms found microplastics transferred up food webs but were not present within predators after 10 days without exposure (Santana et al., 2017). Secondly, the levels of microplastics in fish and other prey species may have been over-estimated due to poor contamination control in some studies (Hermsen et al., 2017). For example, a study of North Sea fish found that 0.25% (1 out of 400) contained microplastics when, as undertaken in our study, strict quality assurance criteria were employed (Hermsen et al., 2017). Lastly, the number of microplastics detected in this study possibly represents a proportion of what is actually present within the marine mammal GITs at the time of death as some may have been lost during the extraction process.

The majority of particles detected in our study were fibres, which corresponds with observations of environmental microplastic concentrations (Claessens et al., 2011; Woodall et al., 2014; Wright et al., 2013b) as well as those found in other studies on cetaceans, turtles and fish (Duncan et al., 2019; Lusher et al., 2013, 2015, 2018; Neves et al., 2015). Similarly, blue and black, the most common colours detected in the marine mammal digestive tracts, frequently dominate composition of particles ingested by turtles, fish and zooplankton (Desforges et al., 2015; Duncan et al., 2019; Lusher et al., 2013; Steer et al., 2017). The mean length of fibres detected in the intestines of a True's beaked whale was 2.16 mm which, again, corresponds closely with the mean length of fibres found in our study (2 mm; Lusher et al., 2015). It is likely that, in our study and others, particles <500 µm in size are under-represented, due to detectability and size of mesh (35 µm) used for vacuum pumping.

In terms of polymer, previous studies found Nylon, polyethylene, polypropylene and polyethylene terephthalate which were also detected in our samples (Besseling et al., 2015; Lusher et al., 2015).

Although a statistical relationship with a modest effect size was found between the cause-of-death category and microplastic abundance, it is not yet possible to draw firm conclusions on the potential biological significance of this observation. More research is required to better understand the potential chronic effects of microplastic exposure on marine mammal health. Sub-lethal effects, from the microplastics themselves or the chemical contaminants present on or within them

are unlikely to be attributable to plastic ingestion at the low levels recorded here. It is not yet known to what extent microplastics act as a vector for transporting these toxicants from the aquatic environment into the tissues of marine mammals. It has been surmised that phthalates could act as a tracer for microplastic ingestion by Mediterranean fin whales (*Balaenoptera physalus*) because high concentrations of these plasticizers were detected in areas that corresponded with the spatial distribution of the whales (Fossi et al., 2012). To date, there is little empirical evidence to demonstrate a direct causal link between chemical contaminant load and microplastic ingestion in marine mammals. Potential health effects, such as depressed immune system function or increased vulnerability to diseases (Desforges et al., 2017; Hall et al., 2006), may not develop until after the microplastics have passed through the body. As a result, a causal relationship between microplastics and sub-lethal effects cannot be ruled out, especially where chronic exposure may lead to the bioaccumulation of toxicants. Additionally, inhalation of atmospheric microplastics (Dris et al., 2016) by marine mammals may be a non-dietary source (Lusher et al., 2018), but the extent to which this occurs is currently unknown. Monitoring of at-sea atmospheric microplastic levels and examination of airways and lungs from stranded animals is needed.

In conclusion, we have shown that at least 10 of the 26 marine mammal species inhabiting or transiting through UK waters are exposed to microplastics through ingestion, though the potential for detrimental impacts is not known. Further examination of larger sample sizes, including investigation of animals of varying feeding strategies (e.g. lunge and suction feeders, such as baleen and beaked whales) in a greater variety of locations is required for comparison. Global hotspots for both large marine vertebrates and plastic pollution, such as the north-west Pacific Ocean (Block et al., 2011; van Sebille et al., 2015), may reveal clearer trends. In addition, investigation into the influence of oceanographic variables, such as currents, on both marine mammal strandings and marine litter may assist our understanding of their spatial relationship.

The methods employed in this study can be applied to a wide range of settings. Here, we were able to set baselines for geographical and temporal comparisons of microplastic ingestion within and across taxa. Exposure to microplastics is likely to be chronic, cumulative and persistent. Although the snapshot provided

by this study cannot yet assess this risk, it does suggest that impacts of microplastic ingestion could manifest in these apex species, and hence further work is needed.

## Methods

*Sample collection*

Post-mortem examinations of 50 stranded marine mammals (Fig. 4, Supplementary Table S5) were carried out by the Scottish Marine Animal Strandings Scheme (SMASS) and the Cetacean Stranding Investigation Programme (CSIP, at the Institute of Zoology and University of Exeter, Penryn campus), during which the gastro-intestinal tracts were extracted and retained for further investigation at Plymouth Marine Laboratory, UK. All post-mortem investigations were conducted using standard procedures(Deaville and Jepson, 2011; Kuiken and Garcia Hartmann, 1991) by experienced marine mammal pathologists in a necropsy facility rated to biosafety level 2. Samples were collected under contract to Defra and the Devolved Governments of Scotland and Wales. All samples were stored at -20°C or below.

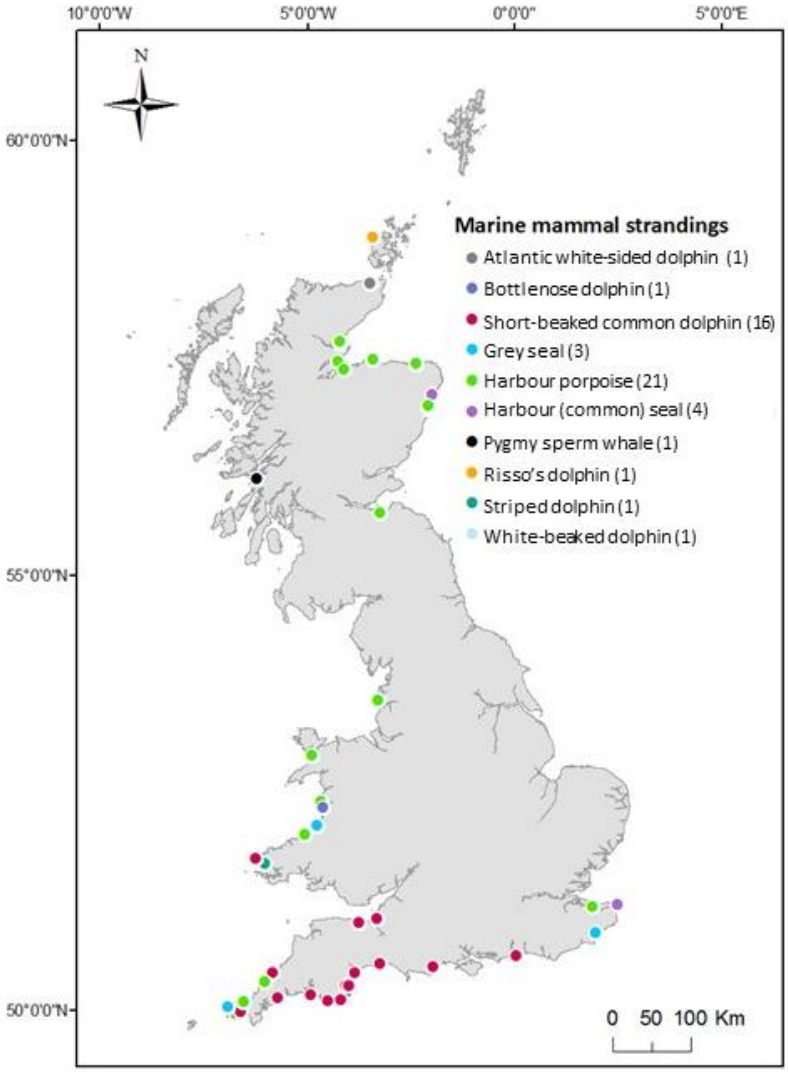


**Fig. 4.** Distribution of marine mammal strandings around the coast of Britain. The coloured points correspond to the marine mammal species (Atlantic white-sided dolphin; *Lagenorhynchus acutus*, bottlenose dolphin; *Tursiops truncatus*, common dolphin; *Delphinus delphis*, grey seal; *Halichoerus grypus*, harbour porpoise; *Phocoena phocoena*, harbour seal; *Phoca vitulina*, pygmy sperm whale; *Kogia breviceps*, Risso's dolphin; *Grampus griseus*, striped dolphin; *Stenella coeruleoalba* and white-beaked dolphin; *Lagenorhynchus albirostris*) and sample size as displayed in the legend. Further details are included in Supplemental Table S5. Map generated using ArcMap 10.3.1.

*Gut content extraction*

The GITs were thawed at room temperature before being rinsed with Milli-Q (ultra-pure, filtered) water to remove any unwanted particles (e.g. sand) adhering

to the external surfaces. In a clean metal tray, each GIT section – intestines and stomach (stomach compartments for cetaceans) - were cut open separately and the inside rinsed with Milli-Q water. The resulting solution was retained in glass beakers. Due to the relatively low amount of organic material present within some stomach compartments (obvious bony parts and otoliths (ear bones) of fish and squid beaks were picked out), it was possible, using a vacuum pump, to pass the content through 35µm mesh discs for later inspection. The intestines (and fore-stomachs of some animals) contained a greater amount of material which could obscure microplastic particles upon visual inspection. Therefore, this material was digested using an enzymatic protocol (see below) to remove organic material whilst retaining inorganic and anthropogenic material for inspection (adapted from Lindeque and Smerdon, (2003)).

*Enzymatic digestion*

Once extracted, the content of the intestines or fore-stomach was placed in a drying oven until the water added during the extraction process evaporated. The dry weight was calculated and the following digestion solution volumes were applied to each 1 g of dried content, the total for each animal varied between 4.5 and 203.5 g. Homogenizing solution (2.2 mL; 400 mM Tris-HCI buffer, 60 mM EDTA, 105 mM NaCl, 1% SDS) was added to the gut content in a clean glass bottle and incubated at 55˚C for 24 hours. A metal spatula was used to physically homogenize the GIT content for 30 seconds, 40 µL of 20 mg $mL^{-1}$ Proteinase K was added and the samples incubated at 55˚C for a further 24 hours. Following this, 400 µL of 5 M sodium perchlorate (NaCLO4) was added and the content physically homogenized for 1 min. Finally, the samples were incubated for 72 hours at 55˚C. Each sample was passed through 35 µm mesh discs (number dependent on amount of material remaining) using a vacuum pump and left to dry at room temperature in a sealed Petri dish.

*Contamination and microplastic loss avoidance*

Extensive measures were implemented throughout to limit the risk of contamination of samples by microplastics present on equipment and air-borne particles within in the atmosphere, see below. As a result, no microplastics were found in the procedural blanks and all controls were clear.

*Gut content extraction*

For health and safety purposes, nitrile gloves and low-density polyethylene (LDPE) fluid protection gowns were worn over a cotton lab coat. Samples of the gloves and gowns were retained to control for any contamination that may have occurred from these sources. Post-mortem examinations were conducted in an ultra-clean facility and the gut content extraction step was performed inside a positive pressure laminar flow hood with the aim of preventing airborne microplastics from settling on the samples. A damp filter paper in a Petri dish was placed within the hood to catch any such particles, allowing for the efficacy of this measure to be assessed. All equipment was thoroughly rinsed with Milli-Q water and all surfaces were wiped down with 70% ethanol prior to any work commencing. All equipment was rinsed with Milli-Q water again between each GIT section. A procedural blank (50 mL Milli-Q water) was run through the process to control for any contamination at this stage.

*Enzymatic digestion*

As above, all equipment was rinsed with Milli-Q and all pipettes and syringes were flushed with Milli-Q prior to use. A procedural blank was run at this stage. Foil lids were used instead of plastic bottle caps as these were previously observed to cause contamination. The metal spatula was rinsed with homogenizing solution (deemed contamination-free after testing) after the homogenising step to avoid loss of particles from samples.

*Vacuum pumping*

Prior to filtering, all mesh discs were visually inspected for potential contamination under a microscope and any particles removed. Milli-Q water was run through the vacuum pump and mesh disc to allow for potential contamination from the equipment to be detected and prevented. If particles were found, the vacuum pump and mesh disc were cleaned again until no particles were detected. Only then were samples filtered. The vacuum pump was then flushed copiously with Milli-Q water to ensure no particles became adhered to the edges and so lost from the sample. The vacuum pump was used inside the laminar flow hood to minimise air-borne contamination. Damp filtered paper inside a petri dish was

placed alongside the samples to control for any contamination that might have occurred.

*Microplastic identification and characterisation*

The mesh discs were visually inspected under an Olympus SZX16 microscope and potential microplastics (identified by colour and uniformity of shape and material; Cole et al., 2014; Norén, 2016) classified by type (fragment or fibre), colour, size and description, and photographed using a microscope mounted Canon EOS 550D DSLR camera. A sub-sample of one particle from each animal ($n$ = 50) was subjected to further analysis using attenuated total reflection-Fourier transform infra-red spectroscopy (ATR-FTIR; PerkinElmer Spotlight 400 FT-IR Imaging System) to confirm the identity of the particles and determine the accuracy level of their visual identification as synthetic materials. Particles were scanned at a resolution of 8 $cm^{-1}$ (wavelength range = 4000 – 650 $cm^{-1}$) and pixel size of 6.25 µm using *SpectrumIMAGE™* software. The resulting spectra were compared to a spectral database from a number of polymer libraries using *Spectrum™* (PerkinElmer). FTIR was attempted for a greater number of particles ($n$ = 65 in total) but obtaining reliable spectra matches was not possible for some due to the extent of degradation. Though these particles were qualitatively similar to those with reliable spectra matches, we were conservative in our inclusion of only particles that exceeded the search score confidence of 0.70 or greater (Lusher et al., 2013) and those considered to have reliable spectra matches (after visual inspection) as this was deemed the most robust method.

*Factors affecting microplastic abundance*

A General Linear Mixed Model (GLMM) was used to examine whether factors such as taxon (cetacean or pinniped), age-class (adult or juvenile), sex (male or female), length of animal and cause-of-death (infectious disease, trauma or other) were related to microplastic abundance. These factors were incorporated within the GLMM as fixed effects and Species was used as a random effect to account for the differing number of animals sampled from each species.

*Distribution of microplastics within GIT*

One-way analysis of variance (ANOVA) was used to assess whether microplastic abundance differs between GIT sections in all animals and among stomach compartments (fore, fundic and pyloric) in cetaceans. Statistical significance was set at a probability level (α) of 0.05. Analyses were undertaken in the statistical computing software, R(R Core Team, 2018).

**Acknowledgements**

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## Supplemental Information

**Table S1.** Model simplification output from generalised linear mixed model (GLMM) – All animals
* Lowest AIC score/ most appropriate model

| Model | AIC-score |
|---|---|
| 1: No. MPs ~ Taxon + Age + Sex + Length + Cause + (1\|Species) | 246.3198 |
| 2: No. MPs ~ Age + Sex + Length + Cause + (1\|Species) | 249.7889 |
| 3: No. MPs ~ Sex + Length + Cause + (1\|Species) | 249.4026 |
| 4: No. MPs ~ Length + Cause + (1\|Species) | 248.7458 |
| 5: No. MPs ~ Cause + (1\|Species) | 239.1526* |
| 6: No. MPs ~ (1\|Species) | 246.745 |

**Table S2.** *P*-values for each fixed effect following removal from generalised linear mixed model (GLMM; ANOVA) – All animals
* Significant *p*-value (<0.05)

| Fixed effects | *p*-value (α) |
|---|---|
| Taxon | 0.0649 |
| Age | 0.9432 |
| Sex | 0.8730 |
| Length | 0.9545 |
| Cause of death | 0.0114* |

**Table S3**. Model simplification output from generalised linear mixed model (GLMM) – Harbour porpoise and common dolphin only
* Lowest AIC score/ most appropriate model

| Model | AIC-score |
|---|---|
| 1: No. MPs ~ Age + Sex + Length + Cause + (1\|Species) | 187.0793 |
| 2: No. MPs ~ Sex + Length + Cause + (1\|Species) | 187.0270 |
| 3: No. MPs ~ Length + Cause + (1\|Species) | 186.8954 |
| 4: No. MPs ~ Cause + (1\|Species) | 178.6772* |
| 5: No. MPs ~ (1\|Species) | 187.7520 |

**Table S4.** *P*-values for each fixed effect following removal from generalised linear mixed model (GLMM; ANOVA) - Harbour porpoise and common dolphin only
* Significant *p*-value (<0.05)

| Fixed effects | p-value (α) |
|---|---|
| Age | 0.8797 |
| Sex | 0.5473 |
| Length | 0.3994 |
| Cause of death | 0.0076* |

**Table S5.** Life-history information for stranded marine mammals and the associated number of microplastics detected

| Sample ID | Year found | Species | Taxa | Age | Sex | Length | Location | Cause of death | Total no. MPs |
|---|---|---|---|---|---|---|---|---|---|
| EX\|C17\|16 | 2016 | Common dolphin | Cetacean | Juvenile | Male | 192 | South-west England | Trauma | 4 |
| EX\|C18\|16 | 2016 | Harbour porpoise | Cetacean | Juvenile | Female | 120 | South-west England | Trauma | 5 |
| EX\|C19\|16 | 2016 | Harbour porpoise | Cetacean | Juvenile | Male | 113 | South-west England | Trauma | 6 |
| EX\|C20\|16 | 2016 | Harbour porpoise | Cetacean | Juvenile | Female | 115.5 | South-west England | Other | 2 |
| EX\|C21\|16 | 2016 | Common dolphin | Cetacean | Adult | Male | 214 | South-west England | Trauma | 1 |
| EX\|C24\|16 | 2016 | Common dolphin | Cetacean | Juvenile | Male | 162 | South-west England | Other | 1 |
| EX\|C28\|16 | 2016 | Common dolphin | Cetacean | Adult | Female | 204 | South-west England | Other | 5 |
| EX\|S9\|16 | 2016 | Grey seal | Pinniped | Juvenile | Female | 90 | South-west England | Infectious disease | 8 |
| M102/16 | 2016 | Harbour porpoise | Cetacean | Juvenile | Male | 104 | Scotland | Infectious disease | 8 |
| M104/16 | 2016 | Harbour porpoise | Cetacean | Juvenile | Female | 111 | Scotland | Trauma | 4 |
| M109/16 | 2016 | Harbour porpoise | Cetacean | Juvenile | Male | 118 | Scotland | Trauma | 7 |
| M126/16 | 2016 | Harbour porpoise | Cetacean | Juvenile | Female | 129 | Scotland | Trauma | 2 |
| M134/16 | 2016 | Harbour porpoise | Cetacean | Juvenile | Male | 118 | Scotland | Other | 3 |
| M150/16 | 2016 | Harbour porpoise | Cetacean | Adult | Female | 160 | Scotland | Other | 7 |
| M157/16 | 2016 | Risso's dolphin | Cetacean | Juvenile | Male | 254 | Scotland | Infectious disease | 9 |
| M178/16 | 2016 | Harbour porpoise | Cetacean | Juvenile | Male | 111 | Scotland | Trauma | 4 |
| M190/16 | 2016 | Harbour porpoise | Cetacean | Juvenile | Male | 125 | Scotland | Trauma | 5 |
| M191/16 | 2016 | Harbour porpoise | Cetacean | Adult | Female | 154 | Scotland | Trauma | 6 |
| M256/11 | 2011 | Pygmy sperm whale | Cetacean | Adult | Male | 211 | Scotland | Other | 4 |
| M267/16 | 2016 | White-beaked dolphin | Cetacean | Adult | Male | 264 | Scotland | Infectious disease | 3 |
| M273/16 | 2016 | Harbour porpoise | Cetacean | Adult | Male | 143 | Scotland | Infectious disease | 11 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| M299/16 | 2016 | Atlantic white-sided dolphin | Cetacean | Adult | Male | 246 | Scotland | Other | 8 |
| M444/14 | 2014 | Harbour seal (Common seal) | Pinniped | Juvenile | Female | 98 | Scotland | Infectious disease | 5 |
| M54/16 | 2016 | Harbour seal (Common seal) | Pinniped | Juvenile | Male | 118 | Scotland | Trauma | 4 |
| SS2015/316 | 2015 | Harbour seal (Common seal) | Pinniped | Adult | Male | 172 | East England | Infectious disease | 7 |
| SS2015/317 | 2015 | Harbour seal (Common seal) | Pinniped | Juvenile | Female | 126 | East England | Other | 1 |
| SS2016/301 | 2016 | Grey seal | Pinniped | Juvenile | Male | 150 | East England | Infectious disease | 4 |
| SS2017/6 | 2017 | Grey seal | Pinniped | Juvenile | Female | 123 | West Wales | Infectious disease | 6 |
| SW2015/341 | 2015 | Harbour porpoise | Cetacean | Juvenile | Female | 104 | West Wales | Trauma | 7 |
| SW2015/422 | 2015 | Striped dolphin | Cetacean | Juvenile | Male | 180 | West Wales | Other | 7 |
| SW2016/210 | 2016 | Harbour porpoise | Cetacean | Adult | Female | 157 | West Wales | Trauma | 4 |
| SW2016/280 | 2016 | Harbour porpoise | Cetacean | Juvenile | Female | 122 | West Wales | Trauma | 2 |
| SW2016/317 | 2016 | Harbour porpoise | Cetacean | Adult | Male | 137 | West England | Other | 4 |
| SW2016/397 | 2016 | Harbour porpoise | Cetacean | Adult | Female | 152 | West Wales | Infectious disease | 10 |
| SW2016/402 | 2016 | Bottlenose dolphin | Cetacean | Juvenile | Male | 145 | West Wales | Trauma | 6 |
| SW2016/411 | 2016 | Common dolphin | Cetacean | Adult | Male | 220 | South-west England | Other | 2 |
| SW2016/416 | 2016 | Common dolphin | Cetacean | Juvenile | Female | 165 | South-west England | Infectious disease | 8 |
| SW2016/446 | 2016 | Common dolphin | Cetacean | Adult | Female | 194 | South-west England | Other | 4 |
| SW2016/447 | 2016 | Common dolphin | Cetacean | Adult | Female | 202 | South-west England | Infectious disease | 7 |
| SW2016/477 | 2016 | Common dolphin | Cetacean | Juvenile | Male | 189 | South-west England | Infectious disease | 3 |
| SW2016/478 | 2016 | Common dolphin | Cetacean | Adult | Male | 207 | South-west England | Infectious disease | 4 |
| SW2016/520 | 2016 | Harbour porpoise | Cetacean | Adult | Male | 138 | East England | Other | 5 |
| SW2016/562 | 2016 | Common dolphin | Cetacean | Adult | Male | 225 | West Wales | Infectious disease | 12 |
| SW2017/12 | 2017 | Common dolphin | Cetacean | Juvenile | Female | 170 | South-west England | Infectious disease | 7 |
| SW2017/13 | 2017 | Harbour porpoise | Cetacean | Adult | Male | 140 | South-west England | Trauma | 2 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| SW2017/15 | 2017 | Common dolphin | Cetacean | Juvenile | Male | 158 | South-west England | Other | 11 |
| SW2017/2 | 2017 | Harbour porpoise | Cetacean | Juvenile | Male | 117 | South-west England | Trauma | 6 |
| SW2017/60 | 2017 | Common dolphin | Cetacean | Juvenile | Female | 177 | South-west England | Other | 9 |
| SW2017/77 | 2017 | Common dolphin | Cetacean | Juvenile | Female | 180 | South-west England | Other | 8 |
| SW2017/8 | 2017 | Common dolphin | Cetacean | Adult | Male | 194 | South-west England | Trauma | 5 |

# Chapter 4: What goes in, must come out: combining scat-based molecular diet analysis and quantification of ingested microplastics in a marine top predator, the grey seal (*Halichoerus grypus*)

This chapter is a reformatted copy of the version submitted for publication in *Methods in Ecology and Evolution*: **Nelms SE,** Parry HE, Bennett KA, Galloway TS, Godley BJ, Santillo D, Lindeque PK (In review) What goes in, must come out: combining scat-based molecular diet analysis and quantification of ingested microplastics in a marine top predator. I conducted all of the sample processing, data analysis and was lead author on this work. PL and HP assisted in the design and implementation of the methodology; KB obtained the samples; DS provided access to essential equipment; all authors contributed critically to the drafts of this manuscript.


## Abstract

Microplastics (plastic particles <5 mm in size) are highly available for ingestion by a wide range of organisms, either through direct consumption or indirectly, via trophic transfer, from prey to predator. The latter is a poorly understood, but potentially major, route of microplastic ingestion for marine top predators. We developed a novel and effective methodology pipeline to investigate dietary exposure of wild top predators (grey seals; *Halichoerus grypus*) to microplastics, by combining scat-based molecular techniques with a microplastic isolation method. We employed DNA metabarcoding, a rapid method of biodiversity assessment, to garner detailed information on prey composition from scats, and investigated the potential relationship between diet and microplastic burden. Outcomes of the method development process and results of both diet composition from metabarcoding analysis and detection of microplastics are presented. Importantly, the pipeline performed well and initial results suggest the frequency of microplastics detected in seal scats may be related to the type of prey consumed. Our non-invasive, data rich approach maximises time and resource-efficiency, while minimising costs and sample volumes required for analysis. This pipeline could be used to underpin a much-needed increase in understanding of the relationship between diet composition and rates of microplastic ingestion in high trophic-level species.

## Introduction

An estimated 9.6 to 25.4 million tonnes of plastic are projected to enter the global ocean annually by 2025 (Jambeck et al., 2015). As a result, improving our understanding of the relationship between plastic pollution and impacts on marine species is a widely acknowledged global priority (UNEP, 2016). Microplastics (plastic particles <5 mm in size) are ubiquitous in many aquatic environments and, due to their small size, are highly bioavailable to a wide-range of species, from low-trophic level organisms to top predators (Desforges et al., 2015; Nelms et al., 2019; Steer et al., 2017).

Marine microplastics, present in seawater, sediment or on vegetation, may be consumed as a result of being mistaken for food or due to indiscriminate feeding strategies (e.g. filter-feeding; Besseling et al., 2015; Hall, Berry, Rintoul, & Hoogenboom, 2015). Additionally, they may be ingested indirectly as a result of trophic transfer, whereby prey containing microplastics are consumed (Farrell and Nelson, 2013; Lourenço et al., 2017; Nelms et al., 2018). Ingestion of microplastics has been found to cause detrimental effects, such as intestinal damage, oxidative stress, energetic depletion and reduced reproductive output in some low trophic-level organisms (Cole et al., 2015; Lei et al., 2018). Furthermore, hydrophobic chemical contaminants present in seawater, such as heavy metals and polychlorinated biphenyls, can adhere to the surface of microplastics and, if ingested, may be released into the organism and exert toxic effects (Teuten et al., 2009).

Understanding predator diets is crucial for examining disruptions to trophic interactions and potential threats to species and habitats that may be caused by anthropogenic factors (Jeanniard-du-Dot et al., 2017), such as plastic pollution. Marine mammals, in particular, are often considered sentinels for marine ecosystem health due to their high trophic-level, extensive foraging ranges, sampling of the full water column and longevity (Bossart, 2011; Fossi et al., 2014; Moore, 2008). Although they ingest microplastics, the route of uptake and resulting biological effects remain unclear (Lusher et al., 2018, 2015; Nelms et al., 2019). For this method development, we chose to focus on a single species (grey seals; *Halichoerus grypus*) as a case study but the pipeline developed here could be applied to any predatory species for which the question of microplastic ingestion is relevant.

Grey seals are top predators in United Kingdom (UK) waters, consuming a range of demersal fish species, such as sand eel, cod and other gadoid fish (Brown et al., 2012; Gosch et al., 2014; Hammond and Wilson, 2016). While it has been shown they can ingest microplastics via trophic transfer from contaminated fish in a captive environment (Nelms et al., 2018), little is known about the extent to which seals ingest microplastics in the wild and whether the risk of doing so relates to their prey composition.

Obtaining dietary information can be difficult for many marine mammal species because they are logistically challenging to access and sample. Stranded animals, from which gut content may be extracted for dietary analysis, are investigated when accessible (Fernández et al., 2014; Mintzer et al., 2008; Nelms et al., 2019). However, animals that died from infectious disease, starvation or other non-trauma related causes of mortality, may introduce bias due to probable abnormal feeding behaviour prior to death (Fernández et al., 2014; Mintzer et al., 2008; Nelms et al., 2019). Grey seals offer the opportunity for relatively easy and representative sample collection because they routinely haul-out on land to rest, breed and moult, during which time they defecate. Although scats (faeces) only provide a snapshot of what the animal has recently consumed (previous ~48 hours), and may be biased toward species present within the immediate proximity of the haul-out site (Grellier and Hammond, 2006; Jeanniard-du-Dot et al., 2017), scat-based methods are non-invasive and have traditionally been utilised to effectively examine diet composition of typical, living animals, using hard-parts from undigested prey remains present in the scat (Grellier and Hammond, 2006; Jeanniard-du-Dot et al., 2017). These methods are, however, labour intensive, time consuming and often miss gelatinous, rare or less robust organisms (Deagle et al., 2009). In addition, different prey species digest at varying rates so their importance in the diet may be under- or over-represented (Grellier and Hammond, 2006; Jeanniard-du-Dot et al., 2017). In recent years, molecular techniques, which can overcome these issues, have been developed using amplification, by Polymerase Chain Reaction (PCR), and sequencing, of a chosen species-specific gene fragment or barcode to better understand diet composition (Deagle et al., 2005). Such a technique, which provides presence/absence information for each potential prey species, can be performed on small quantities of faecal matter, but traditional cloning and subsequent

sequencing of the amplicons is time-consuming and therefore limits the number of scats and sequences that can be processed. Quantitative PCR (qPCR) methods have also been developed to quantitatively assess the presence of a particular species in faecal matter (Matejusová et al., 2008), but this can also be time consuming to develop and uses much smaller amplicons, such that primer design for distinguishing closely related species can be challenging. Both standard and qPCR require some knowledge of the likely prey encountered and the building of an appropriate primer and sequence library to cover all probable prey species (Deagle et al., 2005). Both may also underestimate contribution of species from which the DNA has degraded. More recent tools, such as next generation sequencing, offer a quick and reliable method of assessing diet composition from small sample volumes (McInnes et al., 2017). Metabarcoding is a rapid method of biodiversity assessment that combines two technologies: DNA based identification (barcoding) and high-throughput sequencing (HTS) allowing the mass-amplification (using universal primers) of DNA barcodes from collections of organisms or environmental DNA (Deagle et al., 2018). Such a method yields a greater number of sequences and therefore a greater diversity of prey species without predefining the screening panel (Jeanniard-du-Dot et al., 2017; Thomas et al., 2016), and in addition can provide an estimation of relative abundances in each sample (Albaina, Aguirre, Abad, Santos, & Estonba, 2016; Bucklin & Lindeque, 2016). The use of universal primers designed to amplify a short, highly variable region of DNA enables a large amount of information to be gleaned from degraded DNA, as would be present in faeces (McInnes et al., 2017). In recent years, the expense of HTS has decreased dramatically and metabarcoding is now seen as a powerful and cost-effective tool for assessing diet composition (Berry et al., 2017; Bucklin and Lindeque, 2016).

To date, no studies have examined the direct relationship between diet composition and microplastic ingestion in wild marine mammals. This is important because prey type may be a crucial factor that determines the extent to which plastic is ingested, particularly for top predators for which trophic transfer is potentially the main route of entry (Nelms et al., 2018). Although both metabarcoding and microplastic extraction from faeces/ gut content have been applied separately to a variety of marine and terrestrial taxa, including zooplankton, fish, turtles, birds and marine mammals (metabarcoding; Bucklin &

Lindeque, 2016; Berry et al., 2017; McInnes et al., 2017, microplastics; Cole et al., 2014; Zhao, Zhu, & Li, 2016; Huerta Lwanga et al., 2017; Duncan et al., 2019; Nelms et al., 2019), they usually require different sample processing methods and have not been used concurrently. Here, for the first time, we combine existing DNA extraction techniques for determination of diet composition using molecular scatology methods, with specialist methods designed to isolate microplastics in the same protocol, providing a stream-lined methodology pipeline to assess diet and microplastic abundance simultaneously.

We performed a spiked trial to assess the recovery rate of purpose-made microplastics from seal scats when subjected to two DNA extraction treatments. Using the most appropriate treatment, we extended the full pipeline to 15 wild seal scats from Wales and used metabarcoding to identify the prey composition and relate it to microplastic content. We outline and discuss techniques for overcoming challenges that arise from performing these processes concurrently, such as DNA preservation during microplastic extraction and control of both biological and microplastic contamination. Our aims were to a) develop a technique to combine diet analysis and microplastic quantification; b) provide insights on the diet of a relatively understudied population of grey seals and c) provide recommendations to improve future work linking diet and microplastic burden in marine top predators using scat samples, which may also be applicable to other species and ecosystems.

## Materials and methods

### Sample collection

Grey seal scats (*n* = 15) were collected from a number of haul-out sites (used by unknown individual females and pups) on Skomer Island, Wales (Fig. 1a) in November 2013 (*n* = 9) and October 2014 (*n* = 6), and frozen at -20°C. Analysis was carried out at Plymouth Marine Laboratory, England.

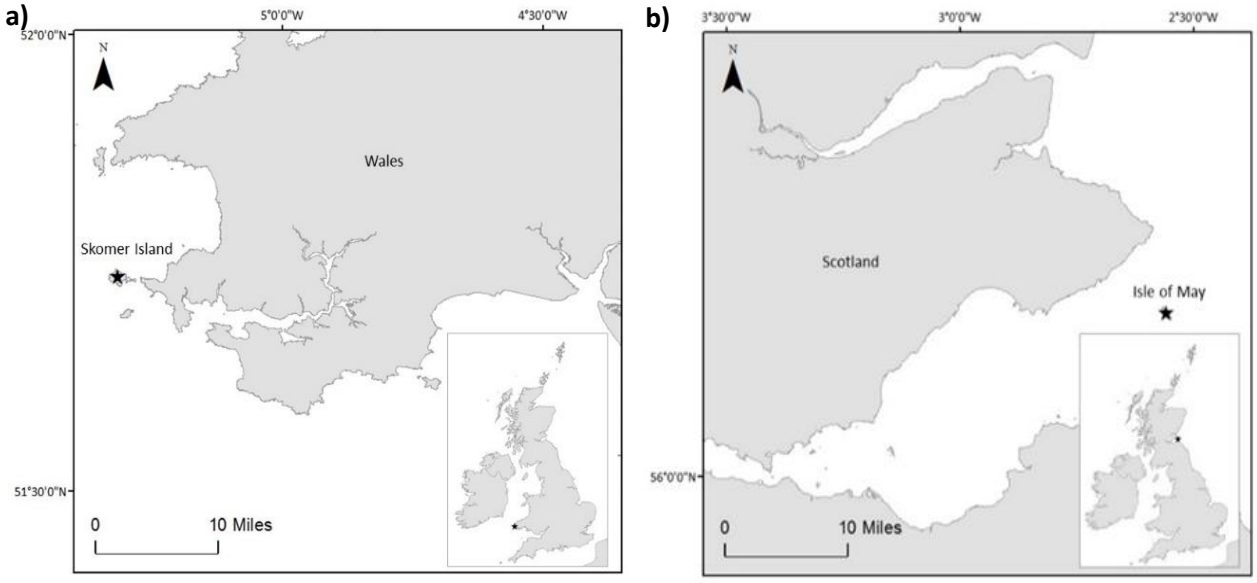


**Fig. 1a)** Scats were collected from haul-out sites on Skomer Island (represented by star), Wales; **b)** Tissue samples from a dead weaned grey seal pup were collected from the Isle of May (represented by star), Scotland.

*Spiked trial*

Two scat sub-samples were spiked with purpose-made microplastics (see below for details) and subjected to different procedures, to develop the optimal protocol for extracting both DNA and microplastics, as outlined below.

*Sample processing*

A scat was thawed and two x 2 g sub-samples were placed into separate sterile centrifuge tubes using a sterile metal spatula. Ten purpose-made microplastics of various types - to represent the diversity found in the marine environment and those which are likely to be encountered by seals and fish (two each of polypropylene, nylon fishing line, fishing rope, low-density polyethylene (LDPE) and expanded polystyrene) were added to each of the two tubes.

*Enzymatic digestion*

To each tube, 15 mL of homogenising solution (400 mM Tris-HCl pH 8, 60 mM EDTA, 150 mM NaCl, 1% SDS) and 500 µL of RNase (10 mg/ mL) were added and the samples incubated at 37°C for 30 mins. Molecular biology grade Proteinase K (14 µL at 250 µg/ mL) was added and samples were incubated for a further 30 mins at 37°C. Sodium perchlorate (4.28 mL of 5 M $NaClO_4$) was

added and the samples shaken at room temperature for 20 mins and incubated at 65°C for a further 20 mins.

*Combined DNA and microplastic extraction procedure comparison*

Two different treatments were applied to the scat sub-samples, hereafter *Treatment A* and *Treatment B* (see Fig. 2), each aimed at combining DNA and microplastic extraction into one procedure;

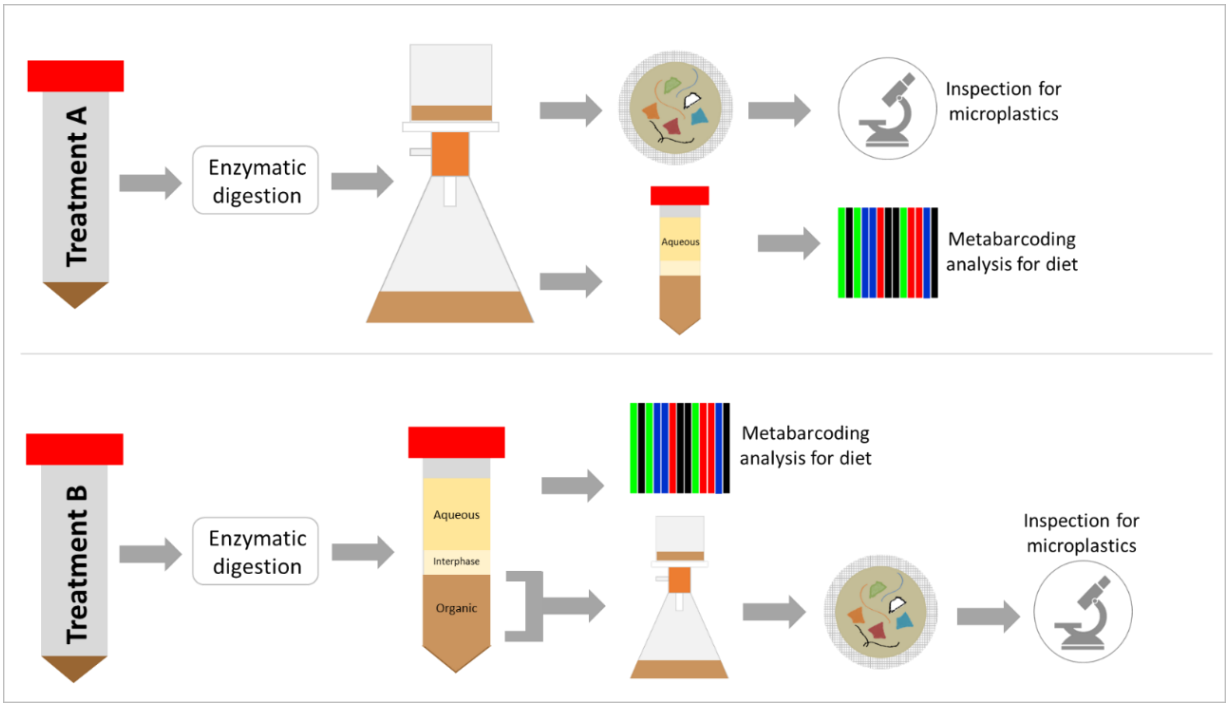


**Fig. 2.** Schematic showing processes applied to Treatments A and B to extract DNA and isolate microplastics.

Treatment A:

- Step 1 - Microplastic removal: Following enzymatic digestion, the entire sample was filtered through a 35 µm mesh disc using a vacuum pump and collected in a sterilised (autoclaved) glass flask. The resulting solution was retained (at room temperature for a minimum amount of time to prevent DNA degradation) for subsequent DNA extraction. The mesh disc containing the scat residue and microplastics was stored in a Petri dish for later microscopic inspection.
- Step 2 - DNA extraction: An equal volume of phenol/chloroform: isoamyl alcohol (24: 1) was added to 15 mL of the scat solution obtained during filtering (Step 1), which was gently mixed by inversion and centrifuged for 5 min (G = 11600). The aqueous phase was removed and an equal volume

of chilled (-20°C) chloroform: isoamyl alcohol (24:1) added to the aqueous phase, which was further separated by centrifugation for another 5 min (G = 11600). The DNA solution (aqueous phase) was removed and precipitated once with 2.5 volumes 100 % ethanol overnight (-20°C) and washed with 70 % ethanol, pelleted using centrifugation, air dried for ~ 3 hours, then re-suspended in 1 mL TE (10 mM Tris, pH 8.0 and 1mM EDTA) buffer overnight.

Treatment B:

- DNA extraction and microplastic removal: Following enzymatic digestion, DNA was extracted using the methods outlined by Step 2 above. However, following separation by phenol/ chloroform:isoamyl alcohol (24:1), the aqueous phase was retained for DNA extraction and only the interphase and organic phase were filtered through a 35 µm mesh using vacuum pump as in Step 1 for microplastic removal above.

*Molecular analysis for diet*

Metabarcoding of DNA in the seal scats, to assess seal diet, was performed by amplification of a region of the 18S nuclear small subunit (nSSU) ribosomal RNA (rRNA) gene and subsequent High Throughput Sequencing (HTS). This method was used because there is at least one variable position in the 18S V9 region, such that metabarcoding of this region can discriminate between species in a reliable way, providing a reference sequence is available in the sequence database (Albaina et al., 2016). First, the quality and quantity of extracted DNA were assessed using a Nanadrop 1000 Spectrophotometer (ThermoScientific, Delaware, USA). Universal primers (Euk_1391f, EukBr; Amaral-Zettler, McCliment, Ducklow, & Huse, 2009) were chosen to target the V9 hypervariable region of the 18S rRNA gene. PCR amplification was performed in triplicate, to reduce PCR bias and increase the likelihood of amplifying rare DNA, 25 µL reactions containing 2.5 µL of each primer (10 µmol/ L), 2.5 µL dNTPs (2 mM), 2.5 units of TaqDNA polymerase (5 units/ µL; Qiagen), 2.5 µL $MgCl_2$ (25mmol/ L), 2.5 µL 10 x buffer, 11 µL molecular grade water and 1 µL DNA extract (range = 0.9 – 42.7 ng/µL). Reactions were amplified through denaturation at 95°C for 2 mins then 27 cycles of (30 s at 95°C, 45 s at 57°C and 45 s at 72°C) followed by a final extension step of 7 mins at 72°C and then stored at 4°C. The PCR products

were checked by gel electrophoresis before being pooled and cleaned up using QIAquick PCR purification kit (Qiagen). Illumina HiSeq high-throughput sequencing was performed by MR DNA (Molecular Research).

*Microplastic quantification*

The dried mesh discs were examined and microplastic particles counted to determine the recovery rate of microplastics used to spike the samples.

*Optimised protocol*

*DNA and microplastic extraction*

Treatment A was used as the pipeline to obtain both diet information and microplastic burden for the 15 wild seal scats.

*DNA sequencing*

Sequencing of the amplified 18S rRNA gene fragments from seal scat was performed at MR DNA (www.mrdnalab.com, Shallowater, TX, USA) on a MiSeq following the manufacturer's guidelines (MiSeq, Illumina). Sequence data were processed using the MR DNA analysis pipeline (MR DNA, Shallowater, TX, USA). In summary, paired end sequences were joined and depleted of barcodes, chimeras and sequences with ambiguous base calls were removed before Operational Taxonomic Units (OTUs) were generated. OTUs were defined by clustering at 3 % divergence (97 % similarity) and any OTUs containing a single sequence were removed. The OTUs were assigned taxonomy using UCLUST (Edgar, 2010), a *de novo* picker within QIIME™ (Quantitative Insights Into Microbial Ecology). A representative set of sequences was then generated and these sequences were assigned taxonomy (at the level of 95 % homology) using the BLASTn search of the NCBI non-redundant dataset. Only OTUs with > 95% homology were retained for further analysis and OTUs assigned as predator DNA (as detailed above), fungi and bacteria were removed.

*Microplastic identification and characterisation*

Following the filtering step, the mesh discs were visually inspected for microplastics using a microscope (Olympus SZX16) and the particles were counted, photographed (microscope mounted Canon EOS 550D DSLR camera),

measured and characterised by type, colour and size. Each potential microplastic was subjected to further analysis to confirm polymer type using attenuated total reflection-Fourier transform infra-red spectroscopy (ATR-FTIR; PerkinElmer Spotlight 400 FT-IR Imaging System). Potential microplastics were scanned at a resolution of 8 $cm^{-1}$ (wavelength range = 4000 – 650 $cm^{-1}$) and pixel size of 6.25 µm using *SpectrumIMAGE™* software. Spectra were compared to a number of polymer libraries using *Spectrum™* (PerkinElmer). Only those considered to have reliable spectra matches (after visual inspection) and a search score confidence of 0.70 or greater (Lusher, McHugh, & Thompson, 2013) were accepted when interpreting output.

*Contamination control*

Strict contamination control measures are essential for studies aimed at assessing microplastic abundance. Though the aims of this study were to develop a methodology rather than produce abundance estimates, best practice contamination control measure were implemented during the handling of samples within the laboratory. Briefly these were; cotton laboratory coats worn at all times, surfaces and equipment thoroughly cleaned with 70% ethanol and/or rinsed with Milli-Q water. The sub-sample of scat for analysis was taken from the centre to avoid any possible contamination of the external surfaces. Microplastics detected in the samples were compared by characteristics (polymer, colour, type) with any plastic equipment used during sample collection, preparation and processing, such as nitrile gloves, polyethylene sample collection bags and Nylon mesh discs. For more details, see (Nelms et al., 2018). For the molecular aspect of this study all equipment was autoclaved following the Milli-Q water rinse to prevent any false positive amplification of DNA.

*Statistical analysis*

The relationship between each of the top three most prevalent prey families (by proportion of sequences) and microplastic abundance was investigated using separate Generalised Linear Models (GLMs). Analyses were undertaken in the statistical computing software, R (GLM; R Core Team, 2018). The distribution of the data was checked for normality using a Q-Q plot and deemed not normal (zero-bounded, asymmetrical). Model selection was performed based on AIC

scores for models with poisson and negative binomial error families and various link function combinations (identity, log and sqrt). Statistical significance was set at a probability level (α) of 0.05.

*Grey seal DNA*

Using HTS methods, prior knowledge of diet composition is not required (as is the case when primers are selected for specific clades) because universal 18S primers allow for the detection of any eukaryote present within scat. It is essential, however, to have a robust reference sequence for the predator species to enable exclusion of these sequences in subsequent analysis. Grey seal 18S was not publicly available for comparison so we generated the sequence information as follows;

*Sample collection*

Tissue samples (liver, kidney and muscle) were taken from a freshly dead, weaned grey seal pup, which had died of natural causes, on the Isle of May, Scotland (Fig. 1b) in December 2017.

*DNA extraction (adapted from Berntson et al., 1999)*

Small sub-samples (5 mm) of tissue were removed and 300 µL of cetyl trimethyl ammonium bromide (CTAB) buffer [2 mL Cetyl trimethyl ammonium bromide 10% in $dH_20$, 2.8 mL 5M NaCl, 0.4 mL 0.5M EDTA (pH 8), 1 mL 1M Tris-Cl (pH 8.0), 0.02 mL B-mercaptoethanol, 3.78 mL $H_2O$] was added. The samples were homogenised using a pestle and mortar and a further 300 µL CTAB buffer was added. Molecular biology grade Proteinase K (1 µL at 20 mg/mL) was added and the samples were further homogenised followed by incubation at 55°C with periodic agitation for 24 hours. An equal volume of cold (-20°C; 24: 1) chloroform: isoamyl alcohol was added, followed by centrifugation at 7700 G for 10 mins. Two volumes of cold (-20°C) 95 % ethanol were added to the aqueous phase and DNA was precipitated for 1 hour at -80°C. The samples were centrifuged at 10,000 G for 30 mins before being washed with cold (-20°C) 70 % ethanol and centrifuged again at 7000 G for 15 mins. The ethanol was then poured off and air-dried for 45 mins, after which the pellets were re-suspended in 50 µL TE and stored at 4°C overnight. The quality and quantity of extracted DNA were assessed

by visualisation using gel electrophoresis (1% agarose) and with a Nanadrop 1000 Spectrophotometer (ThermoScientific, Delaware, USA).

*Sequencing and data processing*

PCR amplification was performed for each tissue type (liver, kidney and muscle; concentration of DNA range = 2823.7 - 5028.9 ng/µL) using the methods and universal primers as described above for seal scat. Following visualisation of the amplification products using gel electrophoresis (2 % agarose gel), DNA extracted from muscle was deemed the most appropriate and reliable for sequencing. Six replicates of the 18S V9 PCR products from grey seal muscle DNA (concentration of DNA range = 0.01 – 0.36 ng/µL) were sequenced in both directions by LGC Genomics, Berlin (Germany). Sequence data from the six replicates were aligned and a consensus sequence generated using MEGA 7 (https://www.megasoftware.net/). The resulting GenBank accession number for grey seal 18S V9 nucleotide sequence is BankIt2148050 seq MH845620 .

## Results

*Spiked trial*

*Observations and microplastic recovery rate*

During the spiked trial, phenol dissolved the purpose-made microplastics and affected the equipment used for filtering, as such Treatment B was not continued. Conversely, Treatment A resulted in a 100% recovery rate of microplastics used to spike the scat and was employed for full analysis of 15 scats.

*Optimised protocol*

*Microplastics*

Microplastics (a total of 17) were found in eight of the 15 subsampled scats (53%), ranging between 1-5 microplastics per scat, as confirmed by FT-IR. Fibres were most commonly detected (76.5%; *n* = 13) while fragments made up 23.5% (*n* = 4). The former ranged from 5.5 mm to 300 µm in length while the latter ranged from 400 µm to 150 µm along the longest edge. The majority were blue (52.9%) followed by red (17.6%), black (11.8%), clear, orange and purple (Fig. 3). The most common polymer type was Nylon (47.1%; *n* = 8) followed by low-density

polyethylene (LDPE), polyethylene terephthalate (PET) and polyethylene (all 17.6%; *n* = 3).

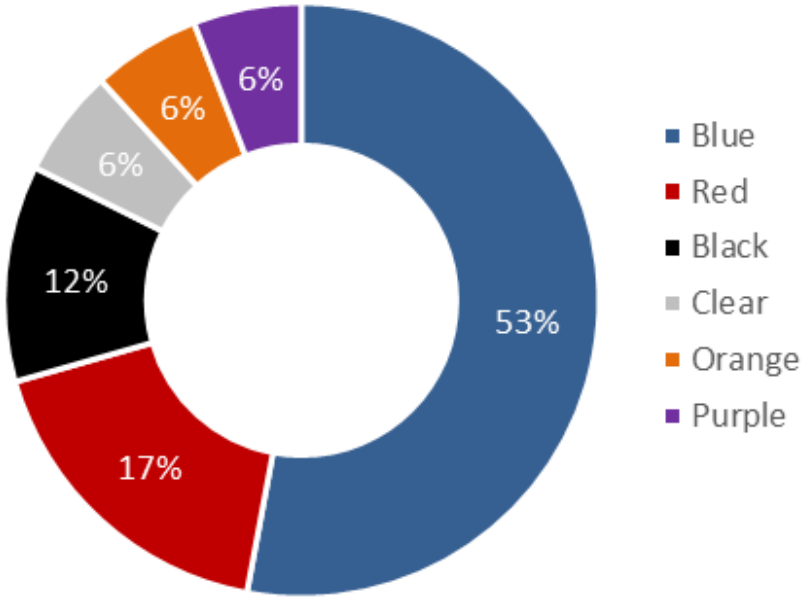


**Fig. 3.** Doughnut plot showing proportions of microplastic colours detected in seal scats (blue = 53%, red = 17%, black = 12%, clear, orange and purple = 12%).

*Seal diet*

In total, 1,449,416 sequences were returned and 9,683 OTUs were formed from the 15 scats. Following the removal of singletons 1,436,089 sequences and 6,993 OTUs remained, of which 353 OTUs were unknowns (< 95% homologous) leaving 6,640 OTUs and 1,432,569 sequences of >95% homology (Table 1). Of these 386,968 (27 %) sequences were assigned as predator (seal) DNA.

**Table 1**. Overall number of OTUs and sequences per Kingdom (eukaryote, fungi, bacteria and viridiplantae) detected in seal scats, and their percentage of the overall composition.

| Kingdom | No. OTUs | Total no. sequences | % composition |
|---|---|---|---|
| Eukaryota | 4881 | 934586 | 65.238 |
| Fungi | 1731 | 495391 | 34.581 |
| Bacteria | 26 | 2579 | 0.180 |
| Viridiplantae | 2 | 13 | 0.001 |
| **Total** | **6640** | **1432569** | **100.000** |

Biological rationale was employed to determine which taxa were subjected to further analysis, based on their likelihood to contain seal prey species, in a stepwise process of taxonomic elimination (Fig. 4). For example, within the Kingdoms listed above, prey are most likely to belong to Metazoans within Eukaryota. Chordata was the most common phylum in this taxon at 71% of sequences, followed by Nematoda (23%) and Cnidaria (5%; Fig.4a). The high proportion of nematodes is likely due to the presence of parasitic worms in the seals' digestive tract, and perhaps other nematode species in the substrata from which the seal scat was collected. Seals are not known to eat Cnidaria and it is likely that their presence reflects the diet of the fish species consumed by the seals. Of the Chordata, mammalian DNA (predator; subsequently removed) was most prevalent (58%) followed by actinopteri (ray-finned-fish; 42% of Chordata and 19% of all sequences returned; Fig. 4b). The three most common families of ray-finned fish were gadidae (specifically Atlantic cod; 47%), pleuronectidae (righteye flounders; 45%) and paralichthyidae (large-tooth flounders; 5%; Fig. 4c). Further details of the prey DNA analysis outputs can be found in Supplemental Information.

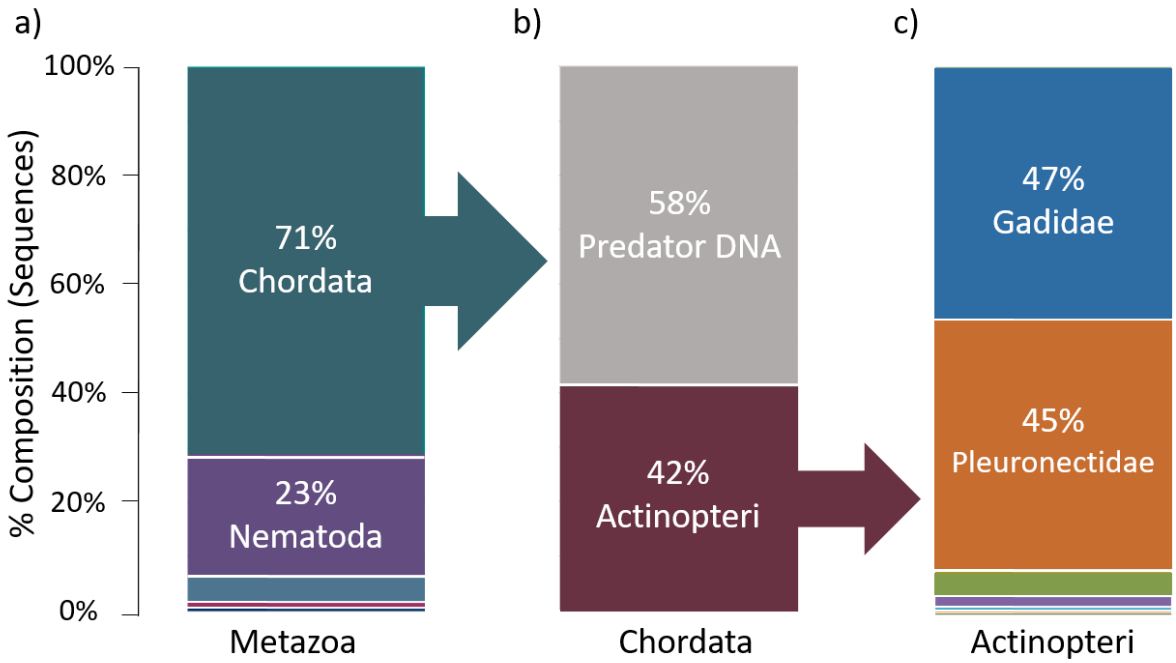


**Fig. 4.** Stepwise process to identify prey a) Percentage sequences by Phyla detected in Metazoa (Chordata (teal; 71%), Nematoda (purple; 23%) and Cnidaria (blue; 5%); b) Percentage sequences by Class detected in Chordata, the most abundant Phyla (predator DNA (grey; 58%) and Actinopteri (burgundy; 42%); c) Percentage sequences by Family (Gadidae (blue; 47%), Pleuronectidae (orange; 45%) and Paralichthyidae (green; 5%) detected in the Actinopteri, the most abundant when predator DNA was eliminated.

*Relationship between prey type and microplastics abundance*

Individual GLMs were run for each prey family and the most appropriate model selected based on AIC scores and *p*-values. A significant positive correlation was found between the proportion of Gadidae and number of microplastics ($F_{1,13}$ = 2.063, $p$ = 0.05, Fig. 5a) whereas a statistically negative (biologically not positive) correlation was observed for the two flounder families (Pleuronectidae $F_{1,13}$ = 0.177, $p > 0.05$; Paralichthyidae $F_{1,13}$ = 10.95, $p < 0.05$; Fig. 5b and c).

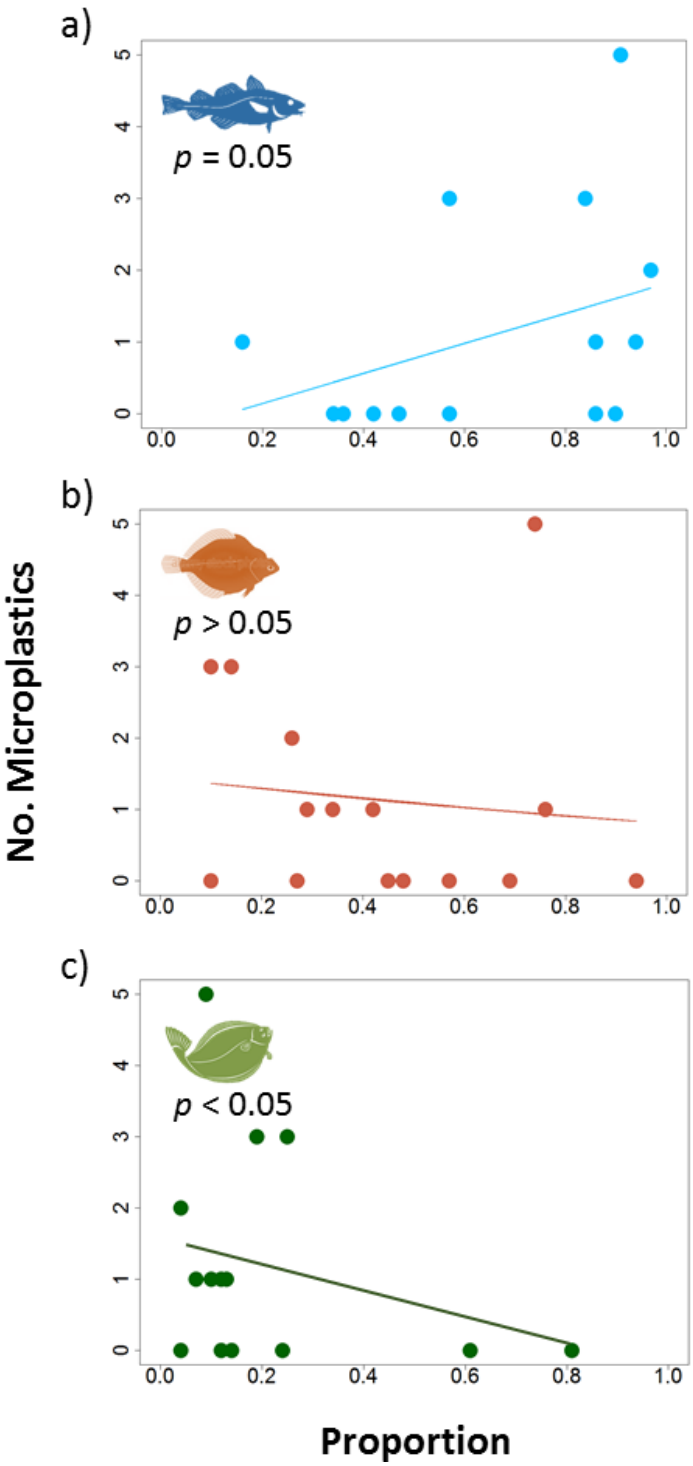


**Fig. 5.** Scatterplots showing the correlation (as investigated using generalised linear models; GLMs) between the number of microplastics and the proportion of the top three most prevalent prey families (by proportion of sequences) **a)** Gadidae **b)** Pleuronectidae **c)** Paralichthyidae.

## Discussion

Marine top predators, such as marine mammals, ingest microplastics (Lusher et al., 2015; Nelms et al., 2019) but the pathways by which this occurs are less well understood. Aside from direct consumption of microplastics from the marine environment, trophic transfer is thought to represent a major route of ingestion for mid and high trophic-level taxa (Hammer et al., 2016; Nelms et al., 2018). Here, we present a novel and effective methodology pipeline that facilitates the simultaneous investigation of a more detailed aspect of trophic transfer – the relationship between specific prey types and the abundance of microplastics detected in scats from wild seals – using small sample volumes. To do so, we used DNA metabarcoding, a powerful molecular technique designed to identify taxonomic groups in complex samples (Bucklin & Lindeque, 2016), combined simultaneously with a microplastic extraction process. We believe that the methods described here could not only advance the development of our understanding of microplastic exposure experienced by these marine top predators, but could also help to elucidate the microplastic contamination status of the wider marine ecosystem by proxy. In addition, as microplastics have been detected in air, soil and freshwater environments (Dris et al., 2016; Huerta Lwanga et al., 2017; Rillig et al., 2017; Windsor et al., 2019), our method could be applied to a wide variety of taxa to investigate this issue across countless ecosystems.

The spiked trial demonstrated that the protocol used for Treatment A produced 100% recovery of purpose-made microplastics, and the extraction of sufficient DNA quantity and quality for metabarcoding analysis. Using this optimal protocol, it was possible to examine the feasibility of assessing prey composition in detail, and detecting microplastics in the scats, concurrently. This stream-lined methodology pipeline removed the necessity of performing both the DNA and microplastic extraction steps separately, which maximised time and resource efficiency and reduced the associated costs and sample required. These outcomes validate the pipeline and demonstrate its efficacy for extracting microplastics and high quality DNA from small volumes of faecal samples, further illustrating its applicability to species other than large marine vertebrates.

Our approach of using the 18S V9 region for metabarcoding diet assessment proved appropriate in the context of seal scats because the amplicon's relatively

small size enabled the analysis of degraded DNA present in faeces, which can be difficult to amplify successfully (McInnes et al., 2017). Additionally, whereas some dietary metabarcoding studies use blocking primers to inhibit the amplification of predator DNA (McInnes et al., 2017; Peters et al., 2015), our methods negate this need, which is beneficial because blocking primers may also prevent amplification of some prey species (McInnes et al., 2017), particularly if the predators and prey are closely related, or if the predator is known to consume conspecifics (Bishop et al., 2016). The use of universal primers to amplify DNA in the gut contents along with predator-specific blocking primers can also introduce biases into the PCR by also blocking amplification of DNA from closely related species and therefore the analysis of predator diets (Piñol et al., 2014). Compared with the traditional approach of using hard-part analysis to examine prey composition, metabarcoding has the ability to detect greater species diversity as well as cartilaginous prey which leave no obvious remains and are unlikely to be detected by eye (Deagle et al., 2009). In addition, a lesser sample volume is required which enables this technique to be used on smaller organisms (Bucklin and Lindeque, 2016). Deriving relative abundance data in diets from metabarcoding can, however, encounter issues such as, primer biases, quality of DNA, differential degradation of material during digestion and heterogeneity in the prey composition of scats (Deagle et al., 2005; Matejusová et al., 2008), so any outputs should be interpreted with these in mind.

Microplastics were detected in over half of the scat sub-samples analysed. There are few other studies on seal scats to compare our results to, but Nelms et al., (2019) found microplastics in the digestive tracts of all wild cetaceans (eight species; 43 individuals) and pinnipeds (two species; 7 individuals) from British waters examined and 1 – 4 microplastics were detected in 48% of scats from captive grey seals fed on wild-caught Atlantic mackerel (*Scomber scombrus*; Nelms et al., 2018). Considering other species, Bråte, Eidsvoll, Steindal, & Thomas (2016) found that 3% of Atlantic cod stomachs from the Norwegian coast contained synthetic polymers and Rummel et al., (2016) detected plastic in approximately 1.2% ($n$ = 2 of 162) of cod and 5.5% ($n$ = 4 of 72) of flounder examined. The finding of greater numbers of microplastics in flounder is contradictory to our results here, in which higher proportions of cod were associated with greater microplastic abundances when compared to the two

flounder families. These observations can be explained by a number of factors. Firstly, as this was a proof of concept study rather than a full environmental assessment, we used a small sample size to develop and test our methodology pipeline. Consequently, any potential relationships detected between prey composition and prey type are likely to be indications only and further work is required to investigate this fully (see methodological recommendations below). Secondly, the methods of examining the presence of microplastics used in the studies above differed from those employed here (i.e. fish digestive tracts vs fish remains from scats) and therefore are likely to yield differing results. Thirdly, spatial variation in microplastic abundance and the overlap with local fish distributions - which also exhibit temporal (e.g. seasonal) and spatial (e.g. regional and depth) variation - may produce diverse patterns and trends. For example, the seals in this study predate fish in the Celtic Sea but the fish examined by Rummel et al., (2016) fed in the North and Baltic Seas where the abundance of microplastics, in both the marine environment and the species that inhabit it, might differ. Though little is known about the diet of grey seals in the Celtic Sea, where Skomer Island is located, a review by Brown, Bearhop, Harrod, & McDonald (2012) revealed that flatfish (e.g. flounders) contribute more to the diet of seals (grey and common; *Phoca vitulina*) in the neighbouring Irish Sea than in all other UK sea areas (Atlantic, North Sea Islands, Moray Firth, southern North Sea) investigated. Similarly Gadoids were a prominent food source in this area (Brown et al., 2012). These findings from hard part analysis correspond to and corroborate the dietary composition reported here obtained through metabarcoding analysis.

Our results are preliminary and not designed to serve as an assessment of microplastic abundance in wild seal diet but as an example of how our protocol could be used to do so accurately, and in a resource and time efficient way, on a larger scale across a wide variety of taxa. We therefore make a number of methodological recommendations to assist in the robust collection and analysis of samples;

1. Wherever possible microplastic contamination should be minimised. Scats should be collected using non-plastic equipment (or scrapings of plastic equipment should be taken for comparison as a control) and a sample from the surrounding substrate should be collected to eliminate any

obvious environmental sources of plastic. During sample processing, a sub-sample from the centre of the scat should be used to avoid any possible contamination of the external surfaces. Further information on contamination control can be found in Nelms et al., (2018).

2. To obtain the best DNA results, and therefore the most accurate representation of prey species present, the collection of fresh scats is optimal (Jeanniard-du-Dot et al., 2017). Additionally, samples should be stored at

   -20°C as soon after collection as possible to prevent DNA degradation (Albaina et al., 2016; Berry et al., 2017; McInnes et al., 2017).
3. To achieve ecologically representative results, we recommend that a systematic and extensive sampling approach be adopted. For example, regular sample collection across informative temporal and spatial scales will allow for any seasonal and geographical variations to be observed. The sample size should also be significantly expanded beyond the 15 analysed for exploratory purposes here.
4. To ground-truth any relationship between microplastic abundance and prey type detected from the scats it would be useful to examine the prey items directly. I.e. sample the gut content of fish species that are known to be consumed by the seals from same area that the scats are collected from. There would also be merit in examining water-borne microplastics and analysing for similarities in fish and scats. This would also reveal whether patterns relating to abundance and type of microplastics as detected in certain fish species, is related to those levels observed within their habitats.

By using non-invasive techniques to assess diet and the presence of microplastics, it is possible to glean insightful information from wild and representative animals, without the need to sample stranded individuals which may not have been feeding normally prior to death, as is often the case in microplastics studies focusing on marine megafauna. Though the methods described here were developed on seal scats, they are applicable to other predatory aquatic taxa where the question of microplastic ingestion may be linked to prey consumption, for which fresh faeces is accessible (such as birds and polar bears, or freshwater vertebrates, e.g. otters); or when gut content can be

extracted from the digestive tract of dead animals, such as cetaceans, elasmobranchs, marine turtles, birds and large predatory fish, for example, tuna. Given that microplastics have been detected in air, soil and freshwater environments (Dris et al., 2016; Huerta Lwanga et al., 2017; Rillig et al., 2017; Windsor et al., 2019), the method developed here could be applied to a wide variety of taxa to investigate the relationship between microplastic ingestion and prey composition in most food web scenarios.

In conclusion, this novel study is the first to combine diet analysis using non-invasive, scat-based molecular techniques and the quantification of ingested microplastics for the purposes of investigating dietary exposure to microplastics in a marine top predator.

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## Supplemental information

**Table S1.** Prey composition from DNA analysis by phyla for Metazoa by number of OTUs, total number of sequences and percentage of overall composition.

| Phylum | No. OTUs | Total no. sequences | % composition |
|---|---|---|---|
| Chordata | 2844 | 662870 | 71.0 |
| Nematoda | 1306 | 210426 | 22.5 |
| Cnidaria | 495 | 43099 | 4.62 |
| Arthropoda | 131 | 12463 | 1.33 |
| Platyhelminthes | 35 | 3175 | 0.34 |
| Ctenophora | 10 | 709 | 0.08 |
| Brachiopoda | 2 | 478 | 0.05 |
| Acanthocephala | 3 | 152 | 0.02 |
| Mollusca | 4 | 106 | 0.01 |
| Annelida | 10 | 88 | 0.0094 |
| Bryozoa | 1 | 4 | 0.0004 |
| **Total** | **4841** | **933570** | **100** |

**Table S2.** Prey composition from DNA analysis by class for Chordata by number of OTUs, total number of sequences and percentage of overall composition.

| Class | No. OTUs | Total no. sequences | % composition |
|---|---|---|---|
| Mammalia | 1128 | 386968 | 58 |
| Actinopteri | 1704 | 274994 | 42 |
| **Total** | **2832** | **661962** | **100** |

**Table S3.** Prey composition from DNA analysis by family for Actinopteri by number of OTUs, total number of sequences and percentage of overall composition.

| Family | No. OTUs | Total no. sequences | % composition |
|---|---|---|---|
| Gadidae | 643 | 128627 | 46.77 |
| Pleuronectidae | 799 | 124156 | 45.15 |
| Paralichthyidae | 130 | 13288 | 4.83 |
| Sparidae | 105 | 5407 | 2.09 |
| Moronidae | 19 | 1571 | 0.57 |
| Sciaenidae | 2 | 1404 | 0.51 |
| Cyprinidae | 2 | 351 | 0.04 |
| Scophthalmidae | 4 | 110 | 0.03 |
| **Total** | **1704** | **274994** | **100** |

# General discussion

Plastic pollution, and its impacts on marine wildlife, is a rapidly expanding and fast-moving field of research (Nelms et al., 2016; Vegter et al., 2014; Senko et al., In review; see Appendix 2). In recent years, there has been growing interest and concern from both the scientific community and general public about its omnipresence within aquatic ecosystems and the potential to cause harm (Cole et al., 2011). Policies aimed at reducing plastic input are slowly being developed but strong and robust evidence is needed to increase our understanding of this pollutant so that suitable and effective strategies are adopted. In this thesis, I examined two main aspects of plastic pollution – sources, trends and patterns of coastal anthropogenic litter and microplastic ingestion by marine mammal top predators. The results of these studies and recommendations for future research are discussed below.

*Overview*

In **Chapter 1**, I explored the composition, distribution and abundance of anthropogenic litter on British beaches using citizen-science data collected by Marine Conservation Society (MCS) volunteers over a 10-year time-period from 736 beaches in England, Scotland and Wales during 3245 beach cleans (Nelms et al., 2017). Although the amount of effort (number of volunteers, length and duration) differed for each beach clean, I was able to correct for this variation and standardise the data, enabling comparisons among beaches. I found that, unsurprisingly, plastic was the most common material and the most frequently found items were large (>2.5 cm) and small (<2.5 cm) plastic fragments, plastic bottle lids, small polystyrene foam pieces and crisp packets. The majority of identifiable items originated from land-based activities, such as public littering. The most polluted beaches were heterogeneously distributed around Britain but there were clear regional differences in litter density – the Western English Channel and Celtic Sea Regional Sea (southwest England and south Wales coasts) had the highest levels of litter, whereas the Scottish Continental Shelf had the lowest. This variation is likely due a combination of factors, such as local population density (permanent residents or tourists), land use and the direction of prevailing winds and currents.

Though no change in overall litter abundance was detected for the 10-year time-period, significant increasing trends were identified for certain items, specifically large fishing net, balloons, polystyrene foam pieces, wet wipes, food packaging and small plastic pieces. In their intended form, these items present issues for both wildlife and humans through ingestion, entanglement, habitat degradation, interactions with maritime equipment and impacts on human health and well-being. Over time, however, they will fragment to form microplastics, thus exposing even more species to the effects of plastic pollution.

*Microplastic trophic transfer in marine top predators*

Microplastics are ingested by animals at all levels of the trophic web, from microscopic zooplankton to marine predators. The latter are exposed to two main ingestion pathways, direct consumption from seawater or substrate, and indirect consumption via trophic transfer, whereby predators consume prey containing microplastics. Until recently, this had only been demonstrated in low trophic level predators, such crabs, and empirical evidence for high trophic-level taxa was lacking. In **Chapter 2**, I sought to fill this knowledge gap by analysing scats from captive grey seals and the wild-caught mackerel they were fed upon. A third of scat subsamples and half of the fish contained between one and four microplastics and the most common polymer for both was ethylene propylene, a synthetic rubber. Since captive seals are not exposed to marine litter like wild seals are, I attributed the presence of microplastics in their scat to trophic transfer from their 'prey'. This demonstrates that trophic transfer could be an indirect, yet major, route of microplastic ingestion for marine top predators (Nelms et al., 2018). Following this investigation, a new question arose – how does diet affect the exposure of wild marine top predators to microplastic ingestion? In **Chapter 4,** I sought to develop a method which would be able investigate this question, by combining scat-based molecular techniques with the microplastic isolation method used in Chapter 2. I employed DNA metabarcoding, a rapid method of biodiversity assessment, to garner detailed information on prey composition from scats, and investigated the potential relationship between diet and microplastic burden. In this preliminary study, I was able to first test two variations of the protocol using a spiked trial, whereby purpose-made microplastics were added to scat samples to assess their recovery rate, before applying the successful

method to 15 (non-spiked) scat subsamples. Though the purpose of this study was to develop a suitable method pipeline, and not to serve as an assessment of microplastic abundance in wild seal diet, the results revealed some interesting initial insights. For example, it appears that there is a relationship between the type of prey species consumed and the number of microplastics present in the scat. This could be due to the foraging behaviour of the prey and its propensity to ingest microplastics, either directly from the marine environment or from its prey. It is hoped that the methods pipeline developed in this study could be applied to a wider and more extensive sampling regime focused on exploring this relationship in greater detail.

*The extent of microplastic ingestion in wild marine mammals*

Although many species of marine mammals are known to ingest anthropogenic debris, little research has been carried out to better understand the extent to which wild marine mammals consume microplastics. In **Chapter 3**, I carried out a comprehensive assessment of the digestive tracts from 50 marine mammals of 10 different species (Atlantic white-sided dolphin, bottlenose dolphin, common dolphin, grey seal, harbour porpoise, harbour seal, pygmy sperm whale, Risso's dolphin, striped dolphin and white-beaked dolphin) that stranded around the British coast (Nelms et al., 2019). I found that every animal contained at least one microplastic but the overall abundance was low. This result is positive in some ways, as it indicates that the animals may be able to expel the synthetic particles, either through excretion or, in the case of cetaceans, regurgitation. It is not clear, however, whether toxicants on (such as hydrophobic persistent chemicals) or in (such as plasticizers) the microplastics are impacting upon animal health, as they are known to leach into biological tissue when ingested. We found a slight but significant relationship between cause of death and microplastic burden, indicating that animals which died of infectious diseases had ingested more microplastics than those that died of trauma or other causes. This may be a sub-lethal effect caused by the microplastics and/ or their associated chemicals, or it may be a result of abnormal feeding behaviour by animals whose health has been previously compromised by an infection. More research is required to better understand the potential chronic effects of microplastic exposure on marine mammal health.

*Future work*

*General marine anthropogenic litter:* Understanding and pinpointing the sources of litter entering aquatic environments is one of the most complex, but critical, steps for minimising plastic pollution and its impacts. It will require the involvement of multiple stakeholders, including policy makers, government officials, designers and innovators, as well as researchers from a variety of disciplines, such as ecology, oceanography, social science, business and politics. Citizen-science data collection is cost-effective and worthwhile but robust and systematic sampling protocols are essential for observing trends and spatial patterns.

*Microplastic ingestion and predator diet*: In Chapters 2 and 4, I investigated microplastic trophic transfer and developed a method which can be used to better understand how it relates to diet composition in marine top predators. Though the methods described here were developed on seal scats, they are applicable to other predatory aquatic taxa where the question of microplastic ingestion may be linked to prey consumption, for which fresh faeces is accessible (such as birds and polar bears, or freshwater vertebrates, e.g. otters); or when gut content can be extracted from the digestive tract of dead animals, such as cetaceans, elasmobranchs, marine turtles, birds and large predatory fish, for example, tuna. In addition, given that microplastics have been detected in air, soil and freshwater environments, the method developed here could be applied to a wide variety of taxa to investigate the relationship between microplastic ingestion and prey composition in most food web scenarios.

*Microplastic ingestion in wild marine mammals*: In Chapter 3, I found that at least 10 of the 26 marine mammal species inhabiting or transiting through UK waters are exposed to microplastics through ingestion. To better understand the global extent of microplastic ingestion, further examination of larger sample sizes, including investigation of animals of varying feeding strategies (e.g. lunge and suction feeders, such as baleen and beaked whales) in a greater variety of locations is required for comparison. Global hotspots for both large marine vertebrates and plastic pollution, such as the north-west Pacific Ocean (Block et al., 2011; van Sebille et al., 2015), may reveal clearer trends. In addition, long-term monitoring may present temporal trends in abundance and type of microplastics ingested. Alternative routes of microplastic intake, such as

inhalation, should also be investigated by examining the lungs and airways of stranded animals.

*Impacts of microplastics on animal health*: Microplastic ingestion has been shown to cause a number of detrimental physiological impacts resulting in a reduction of feeding capacity, energy reserves and reproductive output for smaller low-trophic level organisms (Cole et al., 2013; Sussarellu et al., 2016; Wright et al., 2013a). It is not yet known how it affects larger animals, such as marine mammals. As mentioned above, analysis of a larger sample size with detailed cause of death information may reveal a clearer insight. In addition, closer examination of the digestive tract and its structure may indicate whether any physical damage is inflicted by microplastics, or if very small microplastics/ nanoplastics are able to pass from the intestines into the capillaries and, subsequently, to the liver via the hepatic portal vein portal vein (Pinto da Costa et al., 2016).

The ingestion of microplastics may also represent an additional pathway by which chemical contaminants enter marine mammals, aside from the usual dietary input. Further work is needed to examine the extent to which microplastics increase the exposure of marine mammals to these toxicants and what effects they may present.

*Conclusion*

In summary, plastic pollution is a complicated, trans-boundary and cross-sectoral issue with wide-ranging ecological, social and economic impacts. Marine mammal top predators are exposed to microplastics through two main pathways – direct consumption and trophic transfer. The latter may be an indirect, but potentially major, route of microplastic ingestion. Though ubiquitous in the marine mammals we examined, the low abundance of microplastics suggests they are either excreted or regurgitated. Stomachs, however, do appear to be a site of temporary retention and we do not yet know whether microplastics, or their associated chemicals, cause any detrimental impacts as they pass through. This thesis forms the most detailed assessment of microplastic ingestion in marine mammals to date but the information gathered here represents only a fraction of what is needed to understand this omnipresent pollutant.

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## Appendix 1: Plastic and marine turtles: a review and call for research


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**Plastic and marine turtles: a review and call for research**

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### Abstract

Plastic debris is now ubiquitous in the marine environment affecting a wide range of taxa, from microscopic zooplankton to large vertebrates. Its persistence and dispersal throughout marine ecosystems has meant that sensitivity toward the scale of threat is growing, particularly for species of conservation concern, such as marine turtles. Their use of a variety of habitats, migratory behaviour, and complex life histories leave them subject to a host of anthropogenic stressors, including exposure to marine plastic pollution. Here, we review the evidence for the effects of plastic debris on turtles and their habitats, highlight knowledge gaps,

and make recommendations for future research. We found that, of the seven species, all are known to ingest or become entangled in marine debris. Ingestion can cause intestinal blockage and internal injury, dietary dilution, malnutrition, and increased buoyancy which in turn can result in poor health, reduced growth rates and reproductive output, or death. Entanglement in plastic debris (including ghost fishing gear) is known to cause lacerations, increased drag—which reduces the ability to forage effectively or escape threats—and may lead to drowning or death by starvation. In addition, plastic pollution may impact key turtle habitats. In particular, its presence on nesting beaches may alter nest properties by affecting temperature and sediment permeability. This could influence hatchling sex ratios and reproductive success, resulting in population level implications. Additionally, beach litter may entangle nesting females or emerging hatchlings. Lastly, as an omnipresent and widespread pollutant, plastic debris may cause wider ecosystem effects which result in loss of productivity and implications for trophic interactions. By compiling and presenting this evidence, we demonstrate that urgent action is required to better understand this issue and its effects on marine turtles, so that appropriate and effective mitigation policies can be developed.

**Keywords:** ecosystem effects, entanglement, ghost fishing, ingestion, marine debris, marine turtle, nesting beaches, plastic pollution.

## Introduction

Between 1950 and 2015, the total annual global production of plastics grew from 1.5 million t to 299 million t (PlasticsEurope, 2015). As a result, the abundance and spatial distribution of plastic pollution, both on land and at sea, is increasing (Barnes et al., 2009; Jambeck et al., 2015). Indeed, plastic items have become the principal constituent of marine debris, the majority originating from land-based sources, such as landfill sites, with the remaining deriving from human activities, such as fishing (Barnes et al., 2009; Ivar do Sul et al., 2011). Of particular concern is the longevity of plastic debris and its wide dispersal ability (Barnes et al., 2009; Wabnitz and Nichols, 2010; Reisser et al., 2014b). It has been recorded worldwide in a vast range of marine habitats, including remote areas far from human habitation (Barnes et al., 2009; Ivar do Sul et al., 2011). Transported

across the globe by winds and oceanic currents, high concentrations of floating plastic can accumulate in convergence zones, or gyres, as well as exposed coastlines (Cozar et al., 2014; Reisser et al., 2014b; Schuyler et al., 2014). Enclosed seas, such as the Mediterranean basin, also experience particularly high levels of plastic pollution due to densely populated coastal regions and low diffusion from limited water circulation (Cozar et al., 2015). Once seaborne, plastic persists in the marine environment, fragmenting into smaller pieces as a result of wave action, exposure to UV and physical abrasion (Andrady, 2015). Small particles are highly bioavailable to a wide spectrum of marine organisms (Lusher, 2015). Furthermore, the hydrophobic properties and large surface area to volume ratio of microplastics (fragments of 5 mm in diameter) can lead to the accumulation of contaminants, such as heavy metals and polychlorinated biphenyls (PCBs), from the marine environment. These chemicals, and those incorporated during production (such as plasticizers), can leach into biological tissue upon ingestion, potentially causing cryptic sub-lethal effects that have rarely been investigated (Koelmans, 2015).

For some species, plastics could present a major threat through ingestion, entanglement, the degradation of key habitats, and wider ecosystem effects (Barnes et al., 2009; Vegter et al., 2014; Gall and Thompson, 2015). Among these species are the marine turtles, whose complex life histories and highly mobile behaviour can make them particularly vulnerable to the impacts of plastic pollution (Arthur et al., 2008; Ivar do Sul et al., 2011; Schuyler et al., 2014). As concern grows for the issue of marine plastic and the associated implications for biodiversity, it is essential to assess the risks faced by key species (Vegter et al., 2014). Understanding vulnerability is necessary for setting research priorities, advising management decisions, and developing appropriate mitigation measures (Schuyler et al., 2014; Vegter et al., 2014). This is particularly pertinent given that marine turtles are of conservation concern and often seen as “flagships” for marine conservation issues (Eckert and Hemphill, 2005).

Here, we carry out a comprehensive review of the state of knowledge concerning this anthropogenic hazard and how it impacts marine turtles, and highlight a range of research and innovative methods that are urgently needed. To do so, we searched ISI Web of Knowledge and Google Scholar for the terms plastic, plastic pollution, marine debris, marine litter, ingestion, entanglement,

entrapment, ghost nets and ghost fishing. Plastic and debris were also searched for in conjunction with beach, sand, coral reef, sea grass beds, and fronts. Alongside each search term, we also included the word turtle. We found that the number of peer-reviewed publications per year (between 1985 and 2014) has generally increased over time (Figure 1a) and a descriptive overview of the 64 peer- reviewed studies is given in Table 1 (Ingestion) and Table 2 (Entanglement). We structure our review in five major sections looking at (i) ingestion, (ii) entanglement, (iii) impacts to nesting beaches, and (iv) wider ecosystem effects and then suggest priorities for (v) future research.

**Ingestion**

There are two potential pathways by which turtles may ingest plastic; directly or indirectly. Direct consumption of plastic fragments is well documented and has been observed in all marine turtle species (Carr, 1987; Bjorndal et al., 1994; Hoarau et al., 2014; Schuyler et al., 2014; Figure 2a). Accidental ingestion may occur when debris is mixed with normal dietary items. For instance, one study found that juvenile green turtles (*Chelonia mydas*) consumed debris because it was attached to the macroalgae they target directly (Di Beneditto and Awabdi, 2014). Alternatively, plastic ingestion may be a case of mistaken identity. As turtles are primarily visual feeders, they may misidentify items, such as shopping bags, plastic balloons, and sheet plastic, as prey and actively select them for consumption (Mrosovsky, 1981; Tomas et al., 2002; Gregory, 2009; Hoarau et al., 2014). Hoarau et al. (2014) found a high occurrence of plastic bottle lids in the loggerhead turtles (*Caretta caretta*) they examined and surmised that the lids' round shape and presence floating near the surface visually resemble neustonic organisms normally preyed upon. Laboratory trials have found that turtles are able to differentiate between colours and so the visual properties of plastic are likely to be important factors determining the probability of ingestion (Bartol and Musick, 2003; Swimmer et al., 2005; Schuyler et al., 2012). A number of studies have found that white and transparent plastics are the most readily consumed colours (Tourinho et al., 2010; Schuyler et al., 2012; Camedda et al., 2014; Hoarau et al., 2014). It is not certain, however, whether this trend is a result of selectivity by the turtles or due to the differing proportions of plastic types and colours in the environment (Schuyler et al., 2012; Camedda et al., 2014). Aside

from visual cues, perhaps microbial biofilm formation on plastic debris and the associated invertebrate grazers (Reisser et al., 2014a) cause the particles to emit other sensory cues (such as smell and taste) which could lead turtles to consume them. This, however, remains to be investigated. Indirect ingestion may occur when prey items, such as molluscs and crustaceans that have been shown to ingest and assimilate micro- plastic particles in their tissues (Cole et al., 2013; Wright et al., 2013), are consumed by carnivorous species. Although not yet investigated for marine turtles, trophic transfer has been inferred in other marine vertebrates, specifically pinnipeds (McMahon et al., 1999;

**Table 1.** Summary of all studies on plastic ingestion by marine turtles.

| Species | Ocean Basin | Study area | Reference | Year of Study | *n* | Occurrence % | CCL range* | Pelagic Juvenile | Neritic Juvenile | Adult |
|---|---|---|---|---|---|---|---|---|---|---|
| Loggerhead (*Caretta caretta*) | Mediterranean Sea | Tyrrhenian sea (Tuscany coast) | | 2010-2011 | 31 | 71 | 29.0-73.0 | X | ✓ | ✓ |
| | | Adriatic sea (Croatia, Slovenia) | | 2001-2004 | 54 | 35.2 | 25.0-79.2 | X | ✓ | ✓ |
| | | Central Mediterranean (Sicily) | | 1994-1998 | 44 | 15.9 | unknown | na | na | na |
| | | Central Mediterranean (Italy) | | 2001-2005 | 79 | 48.1 | 25.0-80.3 | X | ✓ | ✓ |
| | | Western Mediterranean (Sardinia) | Camedda *et al.*, 2014 | 2008-2012 | 121 | 14 | 51.38 ± 1.13 | X | ✓ | ✓ |
| | | Western Mediterranean (Balearic archipelago) | Revelles *et al.*, 2007 | 2002-2004 | 19 | 37 | unknown | na | na | na |
| | | Western Mediterranean (Spain) | Tomás *et al.*, 2002 | na | 54 | 75.9 | 34.0–69.0 | ✓ | ✓ | ✓ |
| | | Eastern Mediterranean (Turkey) | Kaska *et al.*, 2004 | 2001 | 65 | 5 | unknown | na | na | na |
| | Atlantic ocean | North–eastern Atlantic (Azores, Portugal) | Frick *et al.*, 2009 | 1986-2001 | 12 | 25 | 9.3–56.0 | ✓ | ✓ | X |
| | | North–western Atlantic (Georgia, USA) | Frick *et al.*, 2001 | na | 12 | 0 | 59.4–77.0 | X | ✓ | ✓ |
| | | North–western Atlantic (Virginia) | Seney and Musick, 2007 | 1983-2002 | 166 | 0 | 41.6-98.5(SCL) | X | ✓ | ✓ |
| | | North–western Atlantic (Florida, USA) | Bjorndal *et al.*, 1994 | 1988-1993 | 1 | 100 | 52 | X | ✓ | X |
| | | Gulf of Mexico (Texas, USA) | Plotkin *et al.*, 1993 | 1986-1988 | 82 | 51.2 | 51.0–105.0 | X | ✓ | ✓ |
| | | Gulf of Mexico (Texas, USA) | Plotkin and Amos, 1990 | 1986-1988 | 88 | 52.3 | unknown | na | na | na |
| | | North-western Atlantic (New York, USA) | Sadove and Morreale, 1989 | 1979-1988 | 103 | 2.9 | unknown | na | na | na |
| | | North–western Atlantic (Florida, USA) | Witherington, 1994 | na | 50 | 32 | 4.03–5.63 | ✓ | X | X |
| | | Gulf of Mexico (Texas & Louisiana, USA) | Cannon, 1998 | 1994 | 20 | 5 | unknown | na | na | na |
| | | South–western Atlantic (Brazil) | Bugoni *et al.*, 2001 | 1997-1998 | 10 | 10 | 63.0-97.0 | X | X | ✓ |
| | Pacific Ocean | South–western (Australia) | Boyle and Limpus, 2008 | na | 7 | 57.1 | 4.6–10.6 | ✓ | X | X |
| | | Central north (Hawaii, USA) | Parker *et al.*, 2005 | 1990-1992 | 52 | 34.6 | 13.5–74.0 | ✓ | ✓ | ✓ |
| | | North-eastern (Shuyak Island, Alaska) | Bane, 1992 | 1991 | 1 | 100 | 64.2 | X | ✓ | X |
| | | North-eastern (California) | Allen, 1992 | 1992 | 1 | 100 | 59.3 | X | ✓ | X |
| | | North-eastern (Baja California, Mexico) | Peckham *et al.*, 2011 | 2003-2007 | 82 | 0 | unknown | na | na | na |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Indian Ocean | South-western (Reunion Islands) | Hoarau *et al.*, 2014 | 2007-2013 | 50 | 51.4 | 68.7 ±4.99 | X | ✓ | ✓ |
| | | North-eastern (Queensland, Australia) | Limpus and Limpus, 2001 | 1989-1998 | 47 | 0 | unknown | na | na | na |
| Green (*Chelonia mydas*) | Mediterranean Sea | Central Mediterranean (Sicily) | Russo *et al.*, 2003 | 1994-1998 | 1 | 0 | 37.8 | X | ✓ | X |
| | Atlantic ocean | South–western Atlantic (Río de la Plata) | González Carman *et al.*, 2014 | 2008-2011 | 64 | 90 | 31.3-52.2 | X | ✓ | X |
| | | South–western Atlantic (Brazil) | Barreiros and Barcelos, 2001 | 2000 | 1 | 100 | 40.5 | X | ✓ | X |
| | | South–western Atlantic (Brazil) | Santos *et al.*, 2011 | 2007-2008 | 15 | 20 | 35.1-60.0 | X | ✓ | X |
| | | South–western Atlantic (Brazil) | da Silva Mendes *et al.*, 2015 | 2008-2009 | 20 | 45 | 33.0-44.0 | X | ✓ | X |
| | | South–western Atlantic (Brazil) | Bugoni *et al.*, 2001 | 1997-1998 | 38 | 60.5 | 28.0-50.0 | X | ✓ | X |
| | | North-western Atlantic (New York, USA) | Sadove and Morreale, 1989 | 1979-1988 | 15 | 6.6 | unknown | na | na | na |
| | | North–western Atlantic (Florida, USA) | Bjorndal *et al.*, 1994 | 1988-1993 | 43 | 55.8 | 20.6-42.7 | X | ✓ | X |
| | | Gulf of Mexico (Texas & Louisiana, USA) | Cannon, 1998 | 1994 | 6 | 33.3 | unknown | na | na | na |
| | | Gulf of Mexico (Texas, USA) | Plotkin and Amos, 1990 | 1986-1988 | 15 | 46.7 | unknown | na | na | na |
| | | South-western Atlantic (Brazil) | Guebert-Bartholo *et al.*, 2011 | 2004-2007 | 80 | 70 | 29-73 | X | ✓ | ✓ |
| | | South-western Atlantic (Brazil) | DiBeneditto and Awabdi, 2014 | na | 49 | 59.2 | unknown | na | na | na |
| | | South-western Atlantic (Brazil) | Tourinho *et al.*, 2010 | 2006-2007 | 34 | 100 | 31.5-56.0 | X | ✓ | X |
| | | South- western Atlantic (Brazil) | Stahelin *et al.*, 2012 | 2010 | 1 | 100 | 39 | X | ✓ | X |
| | | South- western Atlantic (Brazil) | Poli *et al.*, 2014 | 2009-2010 | 104 | 12.5 | 24.0-123.5 | X | ✓ | ✓ |
| | | North–western Atlantic (Florida, USA) | Foley *et al.*, 2007 | 2000-2001 | 44 | 2 | unknown | na | na | na |
| | Pacific Ocean | South–western (Australia) | Boyle and Limpus, 2008 | na | 57 | 54.3 | 5.5-11.3 | ✓ | X | X |
| | | South-eastern (San Andres, Peru) | Quiñones *et al.*, 2010 | 1987 | 192 | 42 | unknown | na | na | na |
| | | South-eastern (Galápagos Islands, Ecuador) | Parra *et al.*, 2011 | 2009-2010 | 53 | 3.3 | 53.0-93.0 | X | ✓ | ✓ |
| | | Central north (Hawaii, USA) | Parker *et al.*, 2011 | 1990-2004 | 10 | 70 | 30.0-70.0 | X | ✓ | ✓ |
| | | North-eastern (Baja California, Mexico) | López-Mendilaharsu *et al.*, 2005 | 2000-2002 | 24 | 0 | unknown | na | na | na |
| | | North-eastern (Gulf of California) | Seminoff *et al.*, 2002 | 1995-1999 | 7 | 29.5 | unknown | na | na | na |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Indian Ocean | North-eastern (Torres Strait, Australia) | Garnett *et al.*, 1985 | 1979 | 44 | 0 | unknown | na | na | na |
| | | North-western (UAE) | Hasbún *et al.*, 2000 | 1997 | 13 | 0 | 35-105.5 | X | ✓ | ✓ |
| | | North-western (Oman) | Ross, 1985 | 1977-1979 | 9 | 0 | unknown | na | na | na |
| Leatherback (*Dermochelys coriacea*) | Mediterranean Sea | Central Mediterranean (Sicily) | Russo *et al.*, 2003 | 1994-1998 | 5 | 40 | 131-145 | X | X | ✓ |
| | Atlantic ocean | North–eastern Atlantic (Gwynedd, Wales) | Eckert and Luginbuhl, 1988 | 1988 | 1 | 100 | 256 | X | X | ✓ |
| | | North–eastern Atlantic (Bay of Biscay) | Duguy *et al.*, 2000 | 1978-1995 | 87 | 55 | unknown | na | na | na |
| | | North–eastern Atlantic (Azores) | Barreiros and Barcelos, 2001 | 2000 | 1 | 100 | 144 | X | X | ✓ |
| | | North-western Atlantic (Sable Island, Nova Scotia) | Lucas, 1992 | 1984-1991 | 2 | 100 | unknown | na | na | na |
| | | North-western Atlantic (New York, USA) | Sadove and Morreale, 1989 | 1979-1988 | 85 | 11.7 | unknown | na | na | na |
| | | South–western Atlantic (Brazil) | Bugoni *et al.*, 2001 | 1997-1998 | 2 | 50 | 135-135 | X | X | ✓ |
| | Pacific Ocean | Central-north Pacific (Midway Island) | Davenport *et al.*, 1993 | 1993 | 1 | 100 | unknown | na | na | na |
| | All | General | Mrosovsky *et al.*, 2009 | 1885-2007 | 408 | 34 | unknown | na | na | na |
| Hawksbill (*Eretmochelys imbricata*) | Atlantic ocean | Gulf of Mexico (Texas, USA) | Plotkin and Amos, 1990 | 1986-1988 | 8 | 87.5 | unknown | na | na | na |
| | | South–western Atlantic (Brazil) | Poli *et al.*, 2014 | 2009-2010 | 15 | 33.3 | 30.9-91.2 | X | ✓ | ✓ |
| | Pacific Ocean | North-eastern (Costa Rica) | Arauz Almengor and Morera Avila, 1994 | 1992 | 1 | 100 | 24.5 | ✓ | x | x |
| Kemp's ridley (*Lepidochelys kempii*) | Atlantic ocean | North-western Atlantic (New York, USA) | Burke *et al.*, 1994 | 1985-1989 | 18 | 0 | unknown | na | na | na |
| | | North-western Atlantic (New York, USA) | Sadove & Morreale 1989 | 1979-1988 | 122 | 0 | unknown | na | na | na |
| | | North–western Atlantic (Florida, USA) | Bjorndal et al. 1994 | 1988-1993 | 7 | 0 | 28.6-66.2 | X | ✓ | ✓ |
| | | Gulf of Mexico (Texas & Louisiana, USA) | Cannon et al. 1998 | 1994 | 167 | 5.4 | unknown | na | na | na |
| | | Gulf of Mexico (Texas, USA) | Plotkin and Amos 1988 | 1986-1988 | 104 | 29.8 | unknown | na | na | na |
| | | Gulf of Mexico (Texas, USA) | Shaver, 1991 | 1983-1989 | 101 | 29 | 5.2-71.0 | ✓ | ✓ | ✓ |
| | | Gulf of Mexico (Texas, USA) | Shaver, 1998 | 1984 | 37 | 19 | unknown | na | na | na |
| Olive ridley (*Lepidochelys olivacea*) | Atlantic ocean | South–western Atlantic (Brazil, Parabia) | Mascarenhas *et al.*, 2004 | 2004 | 1 | 100 | 66 | X | X | ✓ |
| | | South–western Atlantic (Brazil) | Poli *et al.*, 2014 | 2009-2010 | 2 | 100 | 60.0-63.3 | X | ✓ | ✓ |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Flatback (*Natator depressus*) | Indian Ocean | North-eastern (Darwin, Australia) | Chatto, 1995 | 1994 | 1 | 100 | 25.5 | X | ✓ | X |

*CCL = Curved Carapace Length

**Table 2.** Summary of all studies on entanglement in plastic debris by marine turtles.

| Species | Ocean Basin | Study area | Reference | Year of | *n* | CCL | Pelagic | Neritic | Adult | Debris |
|---|---|---|---|---|---|---|---|---|---|---|
| Loggerhead | Atlantic ocean | North–eastern (Boa Vista, Cape Verde | Lopez-Jurado *et al.*, | 2001 | 10 | 62.0- | X | ✓ | ✓ | Fishing |
| | | North–eastern (Terceira Island, Azores) | Barreiros and Raykov, | 2004 - | 3 | 37.3- | X | ✓ | ✓ | Fishing/ |
| | Mediterranean | Tyrrhenian sea (Island of Panarea, | Bentivegna, 1995 | 1994 | 1 | 48.5 | X | ✓ | X | Land- |
| | | Central Mediterranean (Italy) | Casale *et al.*, 2010 | 1980- | 226 | 3.8-97.0 | ✓ | ✓ | ✓ | Fishing/ |
| Green | Indian Ocean | North-eastern (Darwin, Australia) | Chatto, 1995 | 1994 | 1 | 35 | X | ✓ | X | Fishing |
| | | North-eastern (Australia) | Wilcox *et al.*, 2013 | 2005- | 14 | unknown | na | na | na | Fishing |
| Hawksbill | Indian Ocean | North-eastern (Darwin, Australia) | Chatto, 1995 | 1994 | 1 | 32.5 | X | ✓ | X | Fishing |
| | | North-eastern (Australia) | Wilcox *et al.*, 2013 | 2005- | 35 | unknown | na | na | na | Fishing |
| Olive ridley | Indian Ocean | North-eastern (McCluer Island, | Jensen *et al.*, 2013 | unknown | 44 | unknown | na | na | na | Fishing |
| | | North-eastern (Australia) | Wilcox *et al.*, 2013 | 2005- | 53 | unknown | na | na | na | Fishing |
| | | North-eastern (Australia) | Chatto, 1995 | 1994 | 2 | 64 | X | X | ✓ | Fishing |
| | Atlantic Ocean | South-western (Brazil) | Santos *et al.*, 2012 | 1996- | 18 | 2.01- | X | ✓ | ✓ | Fishing |
| Flatback | Indian Ocean | North-eastern (Darwin, Australia) | Chatto, 1995 | 1994 | 1 | 25.5 | X | ✓ | X | Land- |
| | | North-eastern (Australia) | Wilcox *et al.*, 2013 | 2005- | 3 | unknown | na | na | na | Fishing |
| Multiple | Indian Ocean | North-eastern (Australia) | Wilcox *et al*., 2014 | 2005- | 336 | unknown | na | na | na | Fishing |

*CCL = Curved Carapace Length

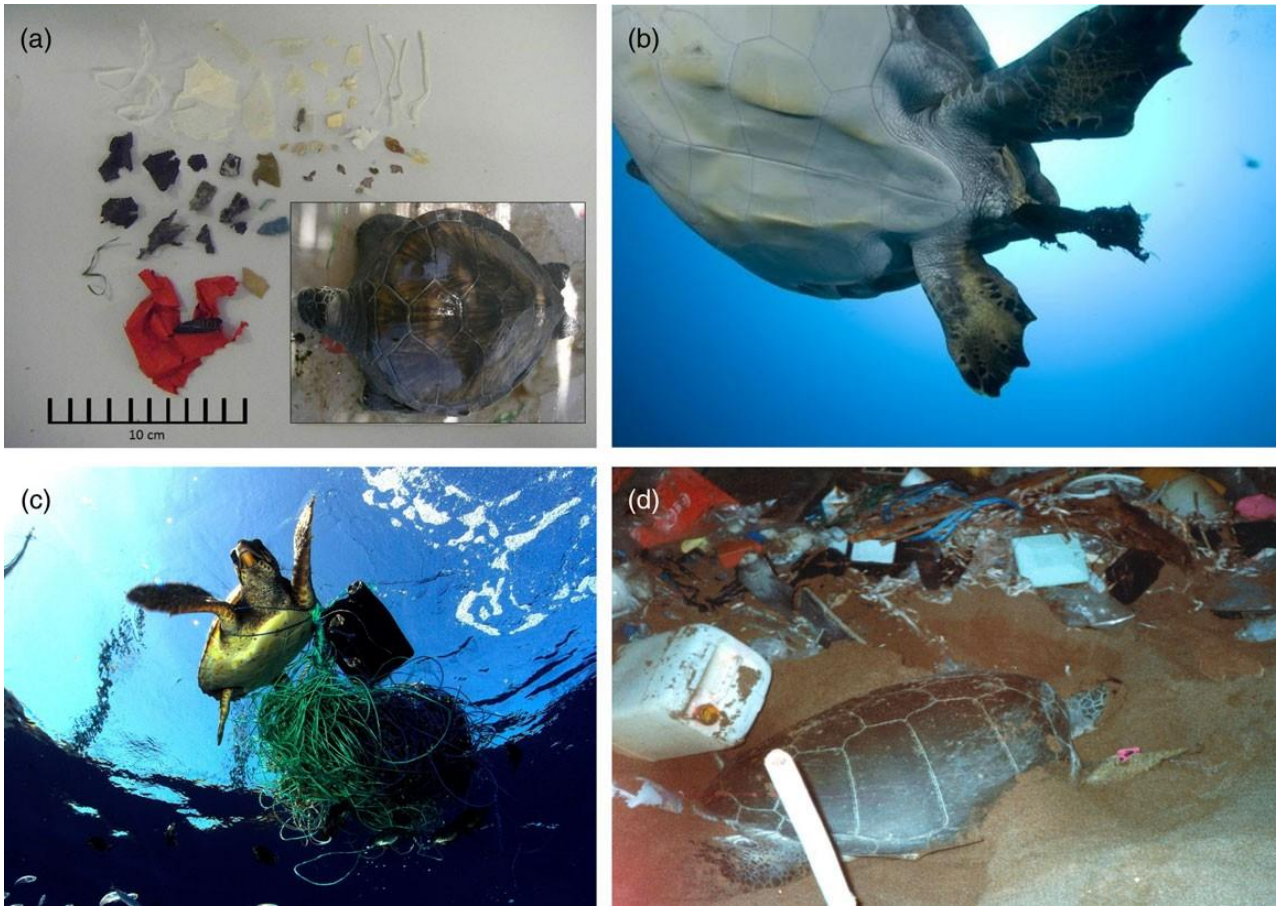


**Figure 2.** Plastics and marine turtles: (a) plastic fragments extracted from the digestive tract of a necropsied juvenile green turtle (inset), found stranded in northern Cyprus (photo: EMD); (b) plastic extruding from a green turtle's cloaca in Cocos Island, Costa Rica (photo: Cristiano Paoli); (c) loggerhead turtle entangled in fishing gear in the Mediterranean Sea (north of Libya) (photo: Greenpeace#/Care`#/Marine Photobank); (d) female green turtle attempting to nest among beach litter, northern Cyprus in 1992 before the commencement of annual beach cleaning (photo: ACB).

Eriksson and Burton, 2003). For example, the prey of the Hooker's sea lion (*Phocarctos hookeri*), myctophid fish, ingest microplastic particles. Subsequently, the otoliths (ear bones) of these fish have been found alongside plastic particles within the sea lion scat, suggesting a trophic link (McMahon et al., 1999). This indirect ingestion may lead to sub-lethal effects that are difficult to identify, quantify and at- tribute to plastic ingestion as opposed to other water quality issues (Baulch and Perry, 2014; Vegter et al., 2014; Gall and Thompson, 2015). These are discussed later in this section. It is likely that feeding ecology and diet, as well as habitat use in relation to areas of high plastic density, determine the likelihood and consequences of plastic ingestion (Bond et al., 2014). These differ among turtle life stages, regional populations and species, meaning that there are likely to be inter- and intraspecies variation in the densities and types of plastic encountered and potentially consumed (Schuyler et al., 2014).

**Life stage**

Both the likelihood of exposure to and consequences of ingestion differ across life stage. Post-hatchlings and juveniles of six of the seven marine turtle species undergo a period of pelagic drifting, known as the "lost year". Although flatback turtles (*Natator depressus*) lack an oceanic dispersal stage, their habitat use during the post- hatchling phase is still likely to be influenced by bathymetry and coastal currents (Hamann et al., 2011). Currents transport hatchlings away from their natal beaches, often to oceanic convergence zones, such as fronts or down-welling areas (Bolten, 2003; Boyle et al., 2009; Scott et al., 2014).

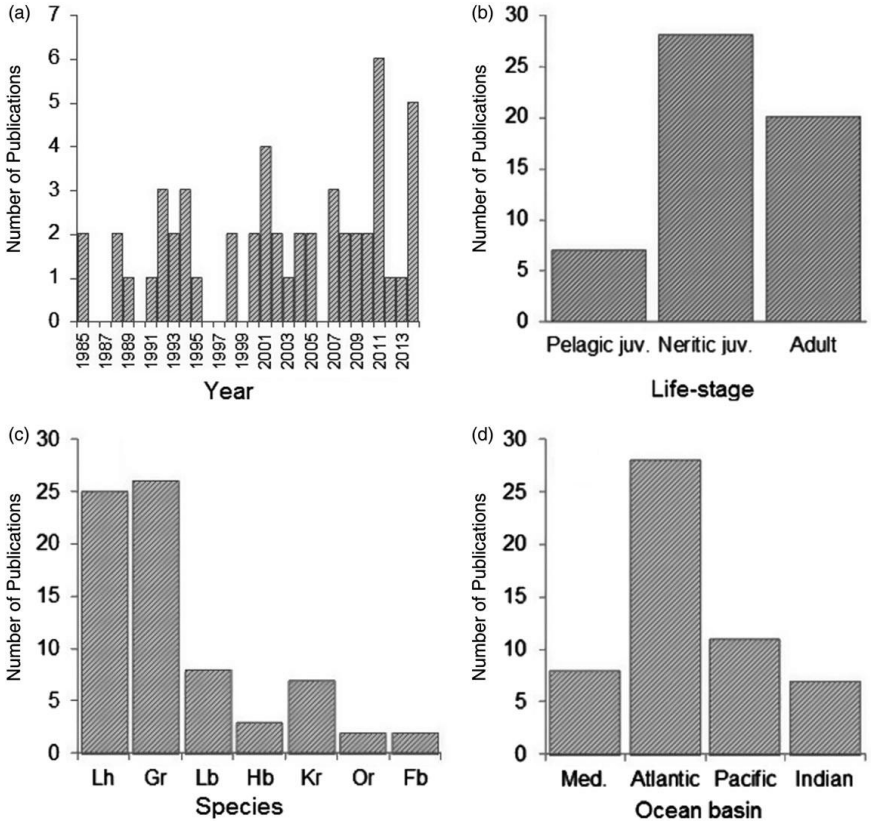


**Figure 1.** Number of publications returned from literature search per (a) year (between 1985 and 2014), (b) life stage, (c) species (Lh, Loggerhead; Gr, Green; Lb, Leatherback; Hb, Hawksbill; Kr, Kemp's ridley; Or, Olive ridley; Fb, Flatback), and (d) Ocean basin.

These areas can be highly productive and act as foraging hotspots for many marine taxa, including fish, seabirds, and marine turtles (Witherington, 2002; Scales et al., 2014; Schuyler et al., 2014). However, along with food, advection also draws in and concentrates floating anthropogenic debris, in- creasing the likelihood of exposure to plastic. This spatial overlap potentially creates an ecological trap for young turtles (Carr, 1987; Tomas et al., 2002; Battin, 2004; Witherington et al., 2012; Cozar et al., 2014). Their vulnerability is further intensified by indiscriminate feeding behaviour, often mistaking plastic for prey items or accidentally ingesting debris while grazing on organisms that are encrusted on such items (McCauley and Bjorndal, 1999; Schuyler et al., 2012; Hoarau et al., 2014). Additionally, turtles in early life history stages, that are small in size, may be at higher risk of mortality from plastic ingestion due to their smaller, less robust, digestive tracts (Boyle, 2006; Schuyler et al., 2012). During our literature search, we found that of all the life stages, young "lost year" juveniles are the most data deficient, but potentially the most vulnerable (Figure 1b). After the post-

hatchling pelagic stage, most populations of chelonid (hard-shelled) species, such as loggerheads, greens, and hawksbills (*Eretmochelys imbricata*), undergo an ontogenetic shift in feeding behaviour where they may switch to benthic foraging in neritic areas (although some populations forage pelagically even in larger size classes; Tomas et al., 2001; Witherington, 2002; Hawkes et al., 2006; Arthur et al., 2008; Schuyler et al., 2012). Some foraging areas experience higher concentrations of plastic debris due to physical processes, for example, frontal systems or discharging rivers, and when such accumulations overlap with turtle foraging grounds, high rates of ingestion may be observed (Gonzalez Carman et al., 2014). Indeed, Gonzalez Carman et al. (2014) reported that 90% of the juvenile green turtles examined had ingested anthropogenic debris and postulated that, aside from aside from the high concentrations of debris, poor visibility (caused by estuarine sediment) and there- fore a reduced ability to discriminate among ingested items may also be a factor.

**Species**

The results from our literature search show that, of all peer-reviewed publications (between 1985-2014; *n* = ~6668) looking at marine turtles, the proportion that investigated occurrences of plastic ingestion is relatively low, ranging from 1-2% depending on species. We found that the majority of these studies focussed on loggerhead (*n* = 24; 44%) and green turtles (*n* = 23; 43%) in contrast to a low number of reports on the leatherback (*Dermochelys coriacea*; *n* = 7, 13%), Kemp's ridley (*Lepidochelys kempii*; *n* = 7; 13%), hawksbill (*n* = 3; 6%), olive ridley (*Lepidochelys olivacea*; *n* = 2; 4%) and flatback turtles (*n* = 2; 4%; Fig. 1c). These biases, however, are broadly reflected by those observed for general turtle studies (green = 35%, loggerhead = 31%, leatherback = 14%, hawksbill = 9%, olive ridley = 5%, kemps ridley = 4% and flatback = 1%). This relationship demonstrates the need for caution when interpreting apparent patterns based on the number of observations of plastic ingestion among species. We also found that the majority of research was carried out in the Atlantic Ocean basin (*n* = 28 of 55 publications on plastic ingestion by turtles; Fig. 1d). These strong biases towards certain species/ regions demonstrate a need to expand research to better understand plastic ingestion for the taxon, globally.

Among marine turtles, there are profound interspecific differences in feeding strategies, diet, and habitat use that could result in varying likelihoods of exposure to

and consequences of plastic ingestion (Bjorndal, 1997; Schuyler et al., 2014). For example, the generalist feeding strategy of loggerhead turtles seems to put them at high risk of ingesting plastic, but their ability to defecate these items, due to a wide alimentary tract, however, demonstrates a certain degree of tolerance (in adults and subadults; Bugoni et al., 2001; Tomas et al., 2001, 2002; Hoarau et al., 2014). This, though, may not mitigate the sub-lethal effects which may occur as a result of plastic ingestion (see the Ecological effects section). Although not heavily studied when compared with the other turtle species (Figure 1c), ingestion rates by Kemp's ridley turtles appear to be low. This may be because they specialize in hunting active prey, such as crabs, which plastic debris is less likely to be mistaken for (Bjorndal et al., 1994). Nonetheless, a potential issue for benthic feeding, carnivorous marine turtle species, such as Kemp's ridley, olive ridley, loggerhead, and flatback turtles, is indirect ingestion of microplastics through consumption of contaminated invertebrate prey, such as molluscs and crustaceans (Parker et al., 2005; Casale et al., 2008) and any associated sediments. Green turtles too are mostly benthic feeders but are largely herbivorous (Bjorndal, 1997). Their preference for sea grass or algae may lead to a greater likelihood of ingesting clear soft plastics resembling their natural food in structure and behaviour. A study in south eastern Brazil found that 59% of juvenile green turtles stomachs contained flexible and hard plastic debris (clear, white, and coloured) and Nylon filaments (Di Beneditto and Awabdi, 2014); another found that 100% of green turtle stomachs examined contained at least one plastic item (Bezerra and Bondioli, 2011). Hawksbills, although omnivorous, prefer to consume sponges and algae, acting as important trophic regulators on coral reefs (Leon and Bjorndal, 2002). While clean-up surveys on coral reefs show that plastic is present in such habitats (Abu-Hilal and Al-Najjar, 2009), data on the ingestion rates and selectivity for hawksbills are lacking (Figure 1c). Peer-reviewed studies investigating ingestion by flatbacks are also scarce, but we found reports that in 2003, a flat- back turtle died following ingestion of a balloon (Greenland and Limpus, 2003) and in 2014, four out of five stranded post-hatchling flatback turtles had ingested plastic fragments (StrandNet Database, 2015). Pelagic species that forage on gelatinous prey, such as leather- backs, are also susceptible to plastic ingestion and Mrosovsky et al. (2009) estimated that approximately one-third of all adult leather- backs autopsied from 1968 to 2007 had ingested plastic. This is thought to be due to similarities to prey items, such as jellyfish, acting as sensory cues to feed (Schuyler et al., 2014).

## Ecological effects

The effects of plastic ingestion can be both lethal and sub-lethal, the latter being far more difficult to detect and likely more frequent (Hoarau et al., 2014; Schuyler et al., 2014; Gall and Thompson, 2015). Tourinho et al. (2010) reported that 100% of stranded green turtles (n = 34) examined in south eastern Brazil had ingested anthropogenic debris, the majority of which was plastic, but the deaths of only three of these turtles could be directly linked to its presence. Damage to the digestive system and obstruction is the most conspicuous outcome and is often observed in stranded individuals (Figure 2b; Camedda et al., 2014). The passage of hard fragments through the gut can cause internal injuries and intestinal blockage (Plotkin and Amos, 1990; Derraik, 2002). Accidental ingestion of plastic fishing line may occur when turtles consume baited hooks (e.g. Bjorndal et al., 1994). As the line is driven through the gut by peristalsis, it can become constricted, causing damage, such as tearing to the intestinal wall (Parga, 2012; Di Bello et al., 2013).

In some cases, the sheer volume of plastic within the gut is noticeable during necropsy or possibly via X-ray or internal examination. Small amounts of anthropogenic debris, however, have been found to block the digestive tract (Bjorndal et al., 1994; Bugoni et al., 2001; Schuyler et al., 2014; Santos et al., 2015). For example, Santos et al. (2015) found that only 0.5 g of debris (consisting of mainly soft plastic and fibres) was enough to block the digestive tract of a juvenile green turtle, ultimately causing its death. Additionally, hardened faecal material has been known to accumulate as a result of the presence of plastic and the associated blockage to the gastrointestinal system (Davenport et al., 1993; Awabdi et al., 2013). On the contrary, it is possible for significant amounts of plastic to accumulate and remain within the gut without causing lethal damage (Hoarau et al., 2014). For example, Lutz (1990) reported that plastic pieces remained in the gut of a normally feeding captive turtle for four months. In the long term, however, a reduction in feeding stimulus and stomach capacity could lead to malnutrition through dietary dilution which occurs when debris items displace food in the gut, reducing the turtle's ability to feed (McCauley and Bjorndal, 1999; Plot and Georges, 2010; Tourinho et al., 2010). Experimental evidence has shown that dietary dilution causes post-hatchling loggerheads to exhibit signs of reduced energy and nitrogen intake (McCauley and Bjorndal, 1999). Post-hatchlings and juvenile turtles are of particular concern because their smaller size means that starvation is likely to

occur more rapidly which has consequences for the turtle's ability to obtain sufficient nutrients for growth (McCauley and Bjorndal, 1999; Tomas et al., 2002).

The presence of large quantities of buoyant material within the intestines may affect turtles' swimming behaviour and buoyancy control. This is especially crucial for deep diving species, such as the leatherbacks (Fossette et al., 2010) and small benthic foragers, such as flatbacks. Additionally, plastic ingestion can compromise a female's ability to reproduce. For example, plastic was found to block the cloaca of a nesting leatherback turtle, preventing the passage of her eggs (Plot and Georges, 2010; Sigler, 2014).

Long gut residency times for plastics may lead to chemical contamination as plasticizers, such as Bisphenol A and phthalates, leach out of ingested plastics and can be absorbed into tissues, potentially acting as endocrine disrupters (Oehlmann et al., 2009). Additionally, due to their hydrophobic properties, plastics are known to accumulate heavy metals and other toxins, such as PCBs, from the marine environment which can also be released during digestion (Cole et al., 2015; Wright et al., 2013). Such contaminants have been shown to cause developmental and reproductive abnormalities in many taxa, such as egg-shell thinning and delayed ovulation in birds as well as hepatic stress in fish (Azzarello and Van Vleet, 1987; Wiemeyer et al., 1993; Oehlmann et al., 2009; Rochman et al., 2013a, b; Vegter et al., 2014). To date, the knowledge base regarding these issues in marine turtles is limited. Indirectly ingested microplastics may pass through the cell membranes and into body tissues and organs where they can accumulate and lead to chronic effects (Wright et al., 2013). The implications of trophic transfer, of both the microplastics and their associated toxins, are as yet unknown (Cole et al., 2013; Wright et al., 2013; Reisser et al., 2014a) and worthy of investigation.

It is possible that the sub-lethal effects of plastic ingestion, including dietary dilution, reduced energy levels, and chemical contamination, may lead to a depressed immune system function resulting in an increased vulnerability to diseases, such as fibropapillomatosis (Landsberg et al., 1999; Aguirre and Lutz, 2004). Stranded juvenile green turtles in Brazil exhibit both high occurrence of plastic ingestion and incidences of this disease (Santos et al., 2011). Additionally, plastic ingestion may impact health and weaken the turtle's physical condition which could impair the ability to avoid predators and survive anthropogenic threats, such as ship strikes and incidental capture by fisheries, issues which already threaten many marine turtle populations

(Lewison et al., 2004; Hazel and Gyuris, 2006; Hoarau et al., 2014). Other longer term consequences could include reduced growth rates, fecundity, reproductive success, and late sexual maturation which could have long-term demographic ramifications for the stability of marine turtle populations (Hoarau et al., 2014; Vegter et al., 2014).

In summary, the potential effects of plastic ingestion on marine turtles are diverse and often cryptic, making it difficult to identify a clear causal link. The sheer scale of possibilities, though, makes this topic one that is in urgent need of further research.

**Entanglement**

Entanglement in marine debris, such as items from land-based sources and lost fishing gear (known as “ghost gear”), is now recognized as a major threat to many marine species (Figure 2c; Gregory, 2009; Wilcox et al., 2013; Vegter et al., 2014). Their sources are difficult to trace, but their widespread distribution indicates that ocean currents and winds may be dispersal factors (Santos et al., 2012; Jensen et al., 2013; Wilcox et al., 2013). Entanglement is one of the major causes of turtle mortality in many areas including northern Australia and the Mediterranean (Casale et al., 2010; Jensen et al., 2013; Wilcox et al., 2013; Camedda et al., 2014). Despite this, quantitative research on mortality rates is lacking and a large knowledge gap exists in terms of implications for global sea turtle populations (Matsuoka et al., 2005). Our literature search returned just nine peer-reviewed publications directly refer- ring to marine debris entanglement and turtles (Bentivegna, 1995; Chatto, 1995; Lopez-Jurado et al., 2003; Casale et al., 2010; Santos et al., 2012; Jensen et al., 2013; Wilcox et al., 2013, 2014; Barreiros and Raykov, 2014) and of these, seven are related to ghost fishing gear. For individual turtles, the effects of entanglement are injuries, such as abrasions, or loss of limbs; a reduced ability to avoid predators; or forage efficiently due to drag leading to starvation or drowning (Gregory, 2009; Barreiros and Raykov, 2014; Vegter et al., 2014). From a welfare perspective, entanglement may cause long-term suffering and a slow deterioration (Barreiros and Raykov, 2014). In some cases, injuries are so severe that amputation or euthanasia are the only options for rehabilitators (Chatto, 1995; Barreiros and Raykov, 2014).

Ghost nets - mostly consisting of synthetic, non-biodegradable fibres, such as Nylon - may persist in the marine environment for many years, indiscriminately “fishing” an undefinable number of animals (Bentivegna, 1995; Wilcox et al., 2013, 2014; Stelfox et al., 2014). Some nets, which may be several kilometres long, drift passively over

large distances (Brown and Macfadyen, 2007; Jensen et al., 2013), eventually becoming bio-fouled by marine organisms and attracting grazers and predators, such as turtles (Matsuoka et al., 2005; Gregory, 2009; Jensen et al., 2013; Stelfox et al., 2014). Although this widespread problem is not unique to turtles, as a taxon, they appear to be particularly vulnerable. For example, a study by Wilcox et al. (2013) reported that 80% of the animals found in lost nets off the Australian coast were turtles. It may be, however, that the physical attributes of marine turtles mean they are more persistent in these nets. For example, their robust carapaces are likely to degrade more slowly and could be easier to identify than carcasses of other marine animals.

More recently, Wilcox et al. (2014) found that nets with large mesh sizes but smaller twine sizes are more likely to entangle turtles, and larger nets seemed to attract turtles, further increasing their catch rates.

Aside from lost or discarded fishing gear, turtles may become trapped in debris from land-based sources. For example, a juvenile loggerhead was found off the island of Sicily trapped in a bundle of polyethylene packaging twine (Bentivegna, 1995) and a juvenile flat- back turtle stranded in Australia after becoming trapped in a woven plastic bag (Chatto, 1995). Reports of such incidences in scientific literature are scarce and it is likely that many individual cases of entanglement are never published (BJG, pers. obs.). Thus, the rates of entanglement in debris, such as sheet plastic and Nylon rope, from land-based sources may be greatly underestimated.

There are few investigations into the susceptibility of the various life stages, but one study found that for olive ridleys, the majority of trapped animals were subadults and adults (Santos et al., 2012). There could be several reasons for this. First, the smaller size of young juveniles enhances their ability to escape. Second, it may be that their carcasses are more readily assimilated into the environment through depredation and decomposition and therefore the evidence of their entanglement is less likely to be discovered. Lastly, it may be that nets are impacting migrating or breeding areas rather than juvenile habitats. The lack of published literature means that the scale of entanglement-induced mortality is unknown, as are the population level impacts of such mortality.

**Impacts on nesting beaches**

Nesting beaches are extremely important habitats for marine turtles and are already under pressure from issues such as sea-level rise and coastal development (Fuentes

et al., 2009). Sandy shorelines are thought to be sinks for marine debris whereby litter, after becoming stranded, is eventually trapped in the substrate or is blown inland (Poeta et al., 2014). As such, various sizes and types of plastic accumulate on marine turtle nesting beaches (Ivar do Sul et al., 2011; Turra et al., 2014). Developed or remote beaches may experience similar levels of contamination but inaccessible beaches, which are not cleaned may experience greater densities of plastic pollution (Figure 2d; Ozdilek et al., 2006; Ivar do Sul et al., 2011; Triessnig, 2012). From large fishing nets to tiny microscopic particles, this debris presents a threat to nesting females, their eggs, and emerging hatchlings (Ivar do Sul et al., 2011; Triessnig, 2012; Turra et al., 2014), further limiting and/or degrading the amount of habitat available for reproduction.

Female marine turtles are philopatric, returning to their natal region to lay eggs in the sand (Bowen and Karl, 2007). Large debris obstacles may impede females during the nest site selection stage, causing them to abort the nesting attempt and return to the sea without depositing eggs (Chaco´n-Chaverri and Eckert, 2007). Alongside this, entanglement is a risk when debris, such as netting, monofilament fishing line, and rope, is encountered (Ramos et al., 2012). Additionally, macro-plastic within the sand column itself may prevent hatchlings from leaving the egg chamber, trapping them below the surface (Authors', pers. obs.).

On emergence from the nest, hatchlings must orient themselves towards the sea and enter the water as quickly as possible to avoid depredation and desiccation (Tomillo et al., 2010; Triessnig, 2012). The presence of obstacles may act as a barrier to this frenzy crawl, not only trapping and killing the hatchlings but increasing their vulnerability to predators and causing them to expend greater amounts of energy (Ozdilek et al., 2006; Triessnig, 2012).

The physical properties of nesting beaches, particularly the permeability and temperature, are known to be altered by the presence of plastic fragments and pellets (Carson et al., 2011). These authors found that adding plastic to sediment core samples significantly increased permeability, and sand containing plastics warmed more slowly, resulting in a 16% decrease in thermal diffusivity (Carson et al., 2011). This, and the fact microplastics have been found up to 2 m below the surface (Turra et al., 2014), indicates potential ramifications for turtle nests. Hatchling sex-ratios are temperature-dependent; consequently, eggs that are exposed to cooler temperatures produce more male hatchlings than females within the clutch (Witt et al., 2010; Carson

et al., 2011; Vegter et al., 2014). Eggs buried beneath sediment containing a high plastic load may also require a longer incubation period to develop sufficiently (Carson et al., 2011). Increased permeability may result in reduced humidity which could in turn lead to desiccation of the eggs (Carson et al., 2011). Other possible impacts include sediment contamination from absorbed persistent organic pollutants or leached plasticizers (Oehlmann et al., 2009; Carson et al., 2011; Turra et al., 2014). For example, the physiological processes of normal gonad development in red-eared slider turtles (*Trachemys scripta*) at male- producing incubation temperatures were altered by PCB exposure, resulting in sex ratios that were significantly biased towards females (Matsumoto et al., 2014).

**Wider ecosystem impacts**

Marine turtles utilize a variety of aquatic habitats that are both neritic and oceanic (Bolten, 2003), but the presence of marine plastics may reduce productivity and cause detrimental changes in ecosystem health (Richards and Beger, 2011). Here, we outline the possible impacts of plastic pollution on two key types of habitats.

*Neritic foraging habitats*

Coral reefs are relied upon by turtles for food, shelter from predators, and the removal of parasites by reef fish at “cleaning stations” (Leo´n and Bjorndal, 2002; Blumenthal et al., 2009; Sazima et al., 2010; Goatley et al., 2012). Richards and Beger (2011) found a negative correlation between the level of hard coral cover and coverage of marine debris as it causes suffocation, tissue abrasion, shading, sediment accumulation, and smothering; all of which may lead to coral mortality (Matsuoka et al., 2005; Brown and Macfadyen, 2007; Richards and Beger, 2011). Additionally, high densities of marine debris appear to impact both the diversity and functioning of coral reef communities, which may lead to a further reduction in biodiversity (Matsuoka et al., 2005; Richards and Beger, 2011). Furthermore, scleractinian corals have been shown to ingest and assimilate microplastics within their tissues, suggesting that high microplastic concentrations could impair the health of coral reefs (Hall et al., 2015). For turtles, changes to these assemblages may lead to a reduced availability of food, a greater predation risk, and an increase in epibiotic loads, such as barnacles (Sazima et al., 2010). Sea grass beds and macroalgae communities are important foraging habitats for the herbivorous green turtle but are sensitive to habitat alterations; the

impacts of which are often observed in the form of reduced species richness (Santos et al., 2011). As highly competitive species become dominant, some marine herbivores are forced to consume less-preferred algal species which in turn reduces the dietary complexity of those organisms (Santos et al., 2011). Balazs (1985) found that this resulted in reduced growth rates of juvenile turtles.

*Oceanic fronts*

As previously discussed, features such as mesoscale thermal fronts and smaller coastal eddies act as foraging hotspots for many marine organisms and are an important micro-habitat for pelagic or surface feeding coastal turtles (Scales et al., 2014, 2015). However, these features are likely sink areas for both macro and microplastics which degrade the quality of these critical habitats, not only in terms of increasing the risk of direct harm through ingestion and entanglement, but by indirectly altering the abundance and quality of the food available (González Carman et al., 2014). Small particles of plastic are known to affect the reproduction and growth rates of low trophic level organisms, for example, zooplankton (Cole et al., 2013). Finally, there is a possibility that the accumulation of such plastic debris can inhibit the gas exchange within the water column, resulting in hypoxia or anoxia in the benthos, which in turn can interfere with normal ecosystem functioning and alter the biodiversity of the seabed (Derraik, 2002).

**Future research**

There are many worthy lines of investigation that would further aid our understanding of the expanding issue of marine plastic pollution and its impact on marine turtles. These are discussed below and summarized in Table

**Table 3.** Summary of recommended research priorities.

| Topic | Methods |
|---|---|
| **Ingestion** | Experiments and field based studies to investigate selectivity (by size, polymer type, colour) and cues leading to ingestion |
| | Targeted efforts to necropsy more widely to address demonstrated geographic, species, life stage, sex and negative-results biases. Incorporate body condition indices. This would be facilitated by global database |
| | Analyse faecal and lavage samples from live specimens with targeted efforts to sample pelagic life stages |
| | Compare data for differences in frequency, amount, type, shape, colour of plastic. Use standardised methods to catalogue debris for comparable results |
| | Create risk maps by assessing exposure to and consequences of ingestion. I.e., utilising satellite tracking, oceanographic and niche modelling in combination with empirical data i.e., from necropsies for ground-truthing |
| | Understand distribution of plastic by size and type in the water column and benthic habitats and develop 3D oceanographic models to understand transport and sink areas for microplastics |
| | *In situ* investigation of plastic passage time and breakdown in turtle gut |
| | Health studies focusing on short and long-term impacts of plastic debris ingestion |
| | Investigate role as secondary consumers including dietary analysis using molecular and isotope techniques. Sample wild invertebrate prey species for the presence of microplastics. Meso-cosm experiments in a controlled laboratory setting |
| | Further investigation of potential for plastic consumption to lead to secondary contamination and methods to detect exposure |
| | Develop methods for the quantification of microplastics in turtle gut content |
| | Develop risk frameworks for species and populations, including detection of vulnerable life stages |
| **Entanglement** | Develop a global online database that records incidents of exposure according to entanglement, debris type, species and life stage |
| | Increase reports and understanding of entanglement in plastic debris from land-based sources |
| | Creating risk maps utilising satellite tracking, oceanographic and niche modelling and data from fisheries layers such as VMS. Ground-truthing and investigation of consequences using empirical data i.e., necropsies |
| | On encountering debris, record the presence/ absence and decomposition state of any entangled turtles |
| | For live strandings, gather information on health status and post-release mortality |

| | |
|---|---|
| **Impacts on nesting beaches** | Record observations of encounters with beach debris for females and hatchlings |
| | Establish baseline surveys for occurrence of plastic debris on beaches with global online database |
| | Sample sand-cores to investigate sub-surface plastic distributions/ densities |
| | Investigate effects on eggs and hatchlings (e.g., sex ratios, embryo development, and fitness) |
| | Use oceanographic modelling to forecast how and when key coastal areas are likely to be impacted by plastic pollution |
| **Ecosystem effects** | Monitor key turtle habitats to generate baseline data. Meso-cosm experiments. Collaborate with other research disciplines and industries |
| | Develop methods to detect and quantify trophic transfer of plastic, associated toxins and bioaccumulation |
| | Explore the impact of plastics on the process of bentho-pelagic coupling |

*Ingestion*

Given the variability in the scale and extent of plastic pollution within the marine environment, there is a clear need to improve our knowledge of relative risk. To achieve this, we advocate for further research to better understand the species, populations, and size classes that have either high likelihood of exposure or high consequences of ingestion. There are a number of biases that need to be eliminated in our knowledge base.

*Geographic*

Studies from the Atlantic are as many as those from all other oceans combined. There clearly needs to be much further work from the Indo-pacific.

*Species*

Although the relative distribution of studies in some way maps to the overall research effort across species, there clearly needs to be more work on species other than loggerhead and green turtles. Of particular interest are hawksbill, leatherback, and olive ridley turtles, given their cosmopolitan distribution and the largely oceanic nature of the latter two species. For Kemp's ridleys and flatbacks, despite their limited geographic range, there is clearly room for a better under- standing of this problem, especially given the conservation status of the former.

*Life stage*

It is suggested that young turtles residing in or transiting convergence zones, where high densities of plastics are known to occur, are at greater risk from ingesting plastic debris. As such, these areas could act as a population sink (Witherington, 2002; Witherington et al., 2012; Gonzalez Carman et al., 2014). As the development and survivorship of young turtles is critical for species persistence, it must be emphasized to generate greater understanding of the impacts of plastics for this life stage and therefore future population viability. Further sampling of frontal zones and knowledge concerning the oceanic developmental stage or "lost years" is also needed. Particularly as the detectability of mortality rates in these post-hatchling turtles is likely to be low (Witherington, 2002; Witherington et al., 2012).

We found only one study that compared ingestion between the sexes, the results of which showed that the frequency of occurrence of debris ingestion was significantly higher in females. Further studies are needed to investigate whether this pattern is observed elsewhere and if so, whether this sex-based difference in plastic ingestion is biologically significant (Bjorndal et al., 1994).

In terms of practical methods for identifying temporal and spatial patterns of plastic ingestion by turtles, Schuyler et al. (2014) found necropsy to be the most effective method. Its application, however, is constrained by small sample sizes because data collection is limited to dead animals. Therefore, every opportunity to examine by-caught and stranded individuals should be utilized (Bjorndal et al., 1994). Alongside gut contents from necropsied turtles, faecal and lavage samples from live specimens should also be analysed. Although not currently a commonly used practise, this may offer insights into survival, partial or total digestion, and comparisons with dead turtles with plastic loads (Witherington, 2002; Hoarau et al., 2014). Integrating body condition indices into necropsy practices will generate a better understanding of the sub-lethal impacts of plastic ingestion, such as malnutrition and the adsorption of toxins (Bjorndal et al., 1994; Gregory, 2009; Labrada-Martagon et al., 2010). It may also be useful to record conditions such as the presence of fibropapillomatosis or epibiotic loads (such as barnacles) as they are also often used as indicators of health (Aguirre and Lutz, 2004; Stamper et al., 2005).

When surveying the literature on plastic debris and marine turtles, it must be emphasized to recognize that published studies do not necessarily represent a randomized sample of the rates of interactions between marine turtles and plastic debris. It is unlikely that researchers who find no evidence of plastic in their study (either in habitats or during necropsies) report negative findings—we found only two studies that did so (Flint et al., 2010; Reinhold, 2015). Data on the absence of marine turtle interactions with plastic debris form an important complement to other datasets, and will facilitate a better understanding of spatio-temporal trends in rates of interactions. We strongly encourage researchers to publish both positive and negative results related to plastics and marine turtles.

We suggest that the endeavours above would be greatly facilitated by a global open access database of necropsy results with regard to plastics. At its simplest, this would be date, location, species, size, state of decomposition, likely cause of death, and some basic descriptors of presence or absence of plastic ingestion or entanglement with associated metadata. This way, workers with a single or small number of cases could still contribute to the global endeavour. Currently, sea-turtle.org hosts a Sea Turtle Rehabilitation and Necropsy Database, STRAND, which allows users to upload gross necropsy reports.

To complement this, it will be important to investigate the passage of plastics through the gut, their degradation, and in addition the transport and bioavailability of bioaccumulative and toxic substances (Campani et al., 2013). Few studies have been con- ducted on the bioaccumulation and trophic transfer of microplastics. Most have focused on invertebrates in controlled laboratory experiments and none focus on the higher trophic level organisms such as marine turtles (Wright et al., 2013). Future studies should sample turtle prey species for the presence of microplastics, examine trophic transfer from prey species containing microplastics, and test for the presence of the contaminants associated with these particles in tissues of necropsied turtles.

To ensure data are comparable, the measurements used to quantify plastic abundance should be standardized. Currently, a variety of metrics are employed, making comparisons among studies difficult. The most common approach is to record total numbers and/or size of fragments. There is a possibility, however, that plastic may break down within the gut or become compressed to appear smaller. Therefore, it is more accurate and comparable to record the total dry weight once extracted (Schuyler et al., 2012; Camedda et al., 2014). Additionally, a wider, more global application of the European Marine Strategy Framework Directive (MSFD) “toolkit” for classification would allow a better comparison of the properties and types of ingested plastics. Furthermore, although not currently included in the MSFD toolkit, efforts to classify colour and/or shape would aid selectivity studies and offer insights as to whether these properties influence the levels of ingestion by turtles (Lazar and Gracan, 2011; Hoarau et al., 2014). The colour and shape should then be compared with those of plastic pieces found in the environment of the species/

life stage investigated. Systematic collection of photos with a scale bar could allow computer-based analytical techniques to be used to classify plastics and compare data across studies.

Debris– turtle interactions often occur in remote locations, far from human habitation and the chronic effects of plastic ingestion may present themselves long after the items were first encountered (Witherington, 2002; Ivar do Sul et al., 2011; Schuyler et al., 2014). The use of tracking technologies, such as satellite telemetry, has already been successfully employed to identify foraging habitats and migration corridors for all sea turtle species. Such data are now being used to develop niche models that can offer a synoptic view of the distribution of a whole segment of a population by season (Pikesley et al., 2013) and can help predict where these ranges may be in the future (Pikesley et al., 2014). Combining such data with plastic debris concentrations using remote sensing methods may identify threat hotspots leading to more effective conservation recommendations (Barnes et al., 2009). At present, the tracking devices used on subadult and adult turtles are not yet available for hatchlings, but technological advances mean they will most likely be available soon as small turtles are now being tracked (Abecassis et al., 2013; Mansfield et al., 2014). In the interim, direct sampling of juveniles in situ with subsequent assessment of plastic loads during a period of captivity would seem a reasonable approach. Alternative methods, such as ocean circulation modelling, can be used to predict the migratory trajectories of hatchling turtles to understand their movements in the open ocean (Putman et al., 2012). Additionally, such methods could also be employed to simulate marine debris dispersal. The development of sophisticated three-dimensional oceanographic models will enable substantial improvements to our understanding of debris transport and turtle movements.

The analysis of trace elements may be used to broadly infer the locations of foraging areas and deduce possible interactions with high concentrations of plastics (Lopez-Castro et al., 2013). A study by Lo´pez-Castro et al. (2013) tentatively identified six oceanic clusters as foraging locations for Atlantic green turtles. As it stands this method needs refinement but with further development, fine-scale mapping may become feasible, offering valuable insights in terms of the spatial overlap with plastic debris distribution.

In addition to the horizontal spatial overlap between turtles and plastics, it would also be beneficial to understand the vertical distribution of quantities and sizes of plastics as this will influence the degree to which marine biodiversity is affected, particularly for those taxa who breathe air and forage near the surface (Reisser et al., 2014b).

*Entanglement*

In a study by Wilcox et al. (2013), the spatial degree of threat posed by ghost net entanglement was predicted by combining physical models of oceanic drift and beach clean data with data concerning marine turtle distributions in northern Australia. This process identified high-risk areas so that recommendations for monitoring and remediation could be made (Wilcox et al., 2013). This approach could be replicated on a global scale but would only be possible where such data exist. As such, a greater research effort is urgently needed (Matsuoka et al., 2005). Indeed, the MSFD Technical Subgroup on Marine Litter is developing a dedicated monitoring protocol for their next report (MSFD GES Technical Subgroup on Marine Litter, 2011). Additionally, fisheries layers, such as vessel monitoring system (VMS) data, may help outline areas of high fishing pressure (Witt and Godley, 2007). To determine the amount of time debris has drifted, Jensen et al. (2013) suggest recording the abundance of epibionts as well as the presence and de- composition state of any entangled turtles.

It would be beneficial to test for any variation in entanglement rates among species and life stages to better understand vulnerability (Wilcox et al., 2013), particularly for small or isolated populations (Jensen et al., 2013). Stranding networks, where dead or alive turtles washed up on beaches are recorded, offer an opportunity to carry out research, not only in terms of debris entanglement but for other anthropogenic issues such as fisheries bycatch and ship strike (Casale et al., 2010). In obvious cases of entanglement, such data can provide valuable insights into the temporal and spatial trends in mortality. However, it can be difficult for the lay- person, and even experts, to confidently determine the cause of death for accurate recording (Casale et al., 2010). For those turtles that strand alive, information should be gathered on health status and post-release mortality. Currently, there are indications that species, time,

depth, and severity of entanglement affect the probability of post-release survival (Snoddy et al., 2009).

During our literature search, we found that the majority of publications on turtle entanglement focus on the issue of ghost fishing by lost gear and few report entrapment in other forms of marine debris, for example, those originating from land-based sources (n ¼ 2 of 9). Exploration into why this may be seems a pertinent next step for research. Additionally, to overcome the lack of peer- reviewed material, efforts should be made to gather and synthesize all relevant grey literature (for example, Balazs, 1984, 1985) in a manner that is suitable for peer-reviewed publication.

As per ingestion, a global open access database of entanglements (and animals discovered without entanglement) would greatly facilitate research efforts.

*Impacts to nesting beach*

Few studies exist whereby the extent of debris-induced mortality, or even interactions, for emerging hatchlings is investigated (Ozdilek et al., 2006; Triessnig, 2012). Observational monitoring programmes could be developed for the many conservation projects operating globally on turtle nesting beaches. This could also be applied to nesting adult females. Currently, most observations are anecdotal (Ozdilek et al., 2006; Triessnig, 2012). Standardized protocols for monitoring and data collection would help facilitate comparisons across studies and over time (Velander and Mocogni, 1999). Additionally, the establishment of a globally accessible data- base of marine debris surveys on nesting beaches would help facilitate an improved understanding of the impacts of plastics on sea turtles that use sandy beaches. Oceanographic modelling could be used to forecast how and when key coastal areas are likely to be impacted in the future.

To date, most studies on coastal microplastic distributions have focused on surface densities. As illustrated by Turra et al. (2014), this may lead to a mis-representation of their overall concentrations. To better quantify this, and develop a greater understanding of the potential impacts on marine turtles and their eggs, three-dimensional sampling should be carried out, investigating the distribution of microplastics at depth (Turra et al., 2014).

Additionally, the relationship between marine plastics and hatchling sex ratios, both in terms of chemical contamination and nest environments, requires greater clarification. This is of interest due to the potential large-scale impacts on turtle populations, particularly as climate change is already predicted to significantly alter female to male ratios (Hawkes et al., 2009).

*Wider ecosystems effects*

Due to the importance of marine habitats such as coral reefs, sea grass beds, and mesoscale thermal fronts for marine turtles, it is essential that we understand the scale of impact from marine debris. Data concerning the distribution and abundance of plastics within these key ecosystems will provide an environmental baseline, a method by which patterns, trends, and, potentially solutions, may be identified. As both coral reefs and seagrass beds are often frequented by divers, utilizing citizen science-based approaches, such as volunteer surveys, may be an affordable and effective method of collecting such data (Smith and Edgar, 2014). Offshore sampling at oceanic fronts may require greater resources but collaboration between research disciplines and industries may help to minimize duplication of effort and expense. As the presence of plastics within the marine environment is of concern not only for biodiversity conservation but for fisheries, tourism, and human health and well-being (through contamination of seafood, a commercially important resource), it is likely that research into this area will grow. As such, it would seem appropriate that those concerned should cooperate to tackle the issue, sharing data where possible.

To better understand the ecosystem level effects of marine plastics, micro- and mesocosm experiments are useful methods of replicating natural environmental systems in controlled conditions (Benton et al., 2007). So far, the majority of such studies have looked only at single taxa, but these study systems allow for investigation into how the links between different marine environments may be affected. As such, further studies should focus on bentho-pelagic coupling to explore the impacts of plastics on the relationships themselves, providing an indication of what influences this foreign debris may have on ecosystem functioning.

## Conclusion

Currently, there is little clear evidence to demonstrate that interactions with plastics cause population level impacts for marine turtles. This, however, should not be interpreted as a lack of effect (Gall and Thompson, 2015). Their widespread distribution, complicated spatial ecology, and highly mobile lifestyles make studying turtles difficult and the development of monitoring programs that deliver statistically robust results challenging. This coupled with the diffuse nature of marine plastic pollution further exacerbates the difficulty in identifying a direct causal link to any potential impacts. In this review, we have demonstrated the widespread and diverse pathways by which plastics may affect turtles. These include ingestion, both directly and indirectly; entanglement; alterations to nesting beach properties; wider ecosystem effects. Although it is evident that this issue could have far-reaching ramifications for marine biodiversity, the lack of focused scientific research into this topic is a major hindrance to its resolution. Policy-makers require robust, comparable, scale-appropriate data (including negative results) on which to develop appropriate and effective mitigation recommendations, something which, as it stands, are severely lacking (Brown and Macfadyen, 2007). We encourage open reporting of plastic– turtle interactions and urge such observations to be sub- mitted for peer-reviewed publication where ever possible. Furthermore, cooperation among scientists, industry, governments, and the general public is urgently needed to confront this rapidly in- creasing form of pollution.

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# Appendix 2: Global impacts of plastic pollution on air-breathing marine megafauna: A review with emerging research priorities

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**Abstract**

Plastic pollution is increasing throughout the world's oceans and is considered to be a major global threat to marine wildlife and ecosystems. It can cause direct mortality to vulnerable marine megafauna, but can also lead to sub-lethal effects that may influence resource acquisition, health, reproductive output, and population growth. Here, we review published effects of plastic pollution on air-breathing marine megafauna (i.e. seabirds, marine mammals, and sea turtles) worldwide, highlight key knowledge gaps, and provide emerging directions for research and management. We found 253 peer-reviewed studies published from 1969–2018 that documented plastic-pollution entanglement (*n*=54), ingestion (*n*=180), or both (*n*=19), 86% of which were undertaken in the Atlantic (54%) and Pacific (32%) Ocean regions. Interactions with plastic pollution were reported for all seven sea turtle species, 53% of seabird species, and 41% of marine mammal species. Seabird ingestion represented 45% of all studies, with loggerhead turtles (*n*=33) and northern fulmars (*n*=25) accounting for the most publications describing entanglement and

ingestion by species. Lethal (16%) and sub-lethal (30%) effects were reported in 46% of studies, and included starvation, drowning, gastrointestinal tract damage, physical injury, constriction, reduced mobility, nutrient dilution, malnutrition, physiological stress, and exposure to toxicants. However, we were unable to find direct evidence of changes in vital rates or abundance trends resulting from these effects. Although the global and pervasive nature of plastic pollution has gained substantial international attention and interest, the actual extent and magnitude of population-level effects on marine megafauna populations worldwide remains largely unknown, representing a major information gap.

## Introduction

Plastic pollution is ubiquitous throughout the world's oceans and can originate from both land- and marine-based sources, such as public littering, sewage and drainage outflows, fisheries, and shipping (Barnes et al., 2009; Ryan et al., 2009; Cózar et al. 2014; Nelms 2017). Marine plastic pollution is increasing globally and accounts for up to 80% of anthropogenic waste accumulated on shorelines and in oceans (Barnes et al. 2009; Nelms et al. 2017). Today, over 5 trillion pieces of plastic, collectively weighing over 250,000 tons, are estimated to be floating in the world's oceans (Eriksen et al. 2014).

Plastic will persist in marine environments due to its chemically engineered durability that makes it resistant to degradation (Barnes et al., 2009; Cózar et al. 2014). It accumulates on shorelines and on the seafloor from shallow waters to deep basins in all major ocean gyres (Thompson et al. 2009; Lebreton et al. 2012; Eriksen et al. 2014; Pham et al. 2014; Woodall et al. 2014), while concentrating at the surface in convergence zones, drift lines, coastal areas, and shores close to beaches where marine wildlife aggregate (Corcoran et al. 2009). Plastic pollution directly affects marine wildlife through ingestion, entanglement, and habitat degradation (Vegter et al. 2014). A recent assessment revealed that 693 species have interacted with marine debris, with plastic accounting for 92% of all interactions (Gall and Thompson 2015).

Air-breathing marine megafauna (i.e. seabirds, marine mammals, and sea turtles) were the taxa most commonly observed to incur effects (Gall and Thompson 2015).

Plastic pollution can cause direct mortality to marine megafauna from entanglement or ingestion, but can also lead to sub-lethal effects that impinge on resource acquisition, health, and other factors (Nelms et al. 2015; Gall and Thompson 2015; Figure 1).


**Figure 1.** Plastic pollution and air-breathing marine megafauna: (a) adult humpback whale (*Megaptera novaeangliae*) found entangled in a conglomerate of ghost fishing gear and other plastic materials, including over 22 different line types, off of Maui, HI, USA (photo: Ed Lyman, NOAA/MMHSRP permit #932-1489); (b) ringed seal (*Phoca hispida*) with plastic strap wrapped around its body (photo: Alaska Fish and Game under ADFG research permits 358-1888 and 358-1787); (c) Laysan albatross (*Phoebastria immutabilis*) chicks and plastic items along a beach at Midway Atoll

National Wildlife Refuge, HI, USA (photo: USFWS); (d) Laysan albatross chick with ingested plastic after its death at Midway Atoll National Wildlife Refuge, HI, USA (photo: John Klavitter, USFWS); (e): three olive ridley turtles (*Lepidochelys olivacea*) entangled at the surface in a conglomerate of ghost fishing gear in the Maldives. Massive conglomerates of ghost gear tangled together are common in the Maldives during the NE Monsoon, where turtles frequently become entangled in a single conglomerate of ghost gear (photo: Dave Bretherton/Olive Ridley Project); (f) surface convergence front in the northern Gulf of Mexico, with accumulated sargassum algae and a typical plastic load. These surface-front "weedlines" are important oceanic habitat for marine megafauna including turtles, seabirds, and cetaceans (photo: Blair Witherington).

Marine megafauna are particularly susceptible to plastic pollution because of their broad distributions, complex life histories that expose many species to impacts at sea and on land, and high trophic levels. Ingestion frequency has been increasing globally in seabirds and sea turtles since the 1960s (Robards et al. 1995; Ryan et al. 2009; Teuten et al. 2009) and mid 1980s (Schuyler et al. 2014a), respectively. Recent estimates suggest that 99% of all seabird species and 95% of the individuals within these species may have plastic in their digestive tracts by 2050 (Wilcox et al. 2015). Similarly, 52% of individual sea turtles may have already ingested macroplastic (Schuyler et al. 2015), with microplastics now ubiquitous in this taxon (Duncan et al. 2018). However, despite increases in marine megafauna interactions with plastic pollution, the effects on individuals and populations relative to impacts of other threats have not been thoroughly investigated.

In this review, we systematically compiled and evaluated available information from published studies on plastic pollution research conducted on seabirds, marine mammals, and sea turtles worldwide. We first assess the current state of the peer-reviewed literature regarding interactions between plastic pollution and marine megafauna, which includes an evaluation of the taxonomic and spatial extent of types of lethal and sub-lethal effects and their potential for individual and population-level impacts. We then provide an overview of the different pathways by which marine megafauna interact with plastic pollution. We conclude by highlighting critical

knowledge gaps, future research priorities, and recommendations for mitigation.

## Methods

We conducted an extensive literature review to assess available information on impacts of plastic pollution on air-breathing marine megafauna. We searched ISI Web of Knowledge from 1969 to 2018 for the terms *plastic*, *litter*, *debris*, and *ingest/entangle*. Alongside each search term, we also incorporated the relevant taxon of interest including *marine mammal*, *cetacean*, *whale*, *dolphin*, *porpoise*, *seal*, *sea lion*, *walrus*, *dugong*, *manatee*, *sea cow*, *sea otter*, *turtle*, *seabird*, and *marine bird*. Studies were evaluated and filtered to remove cases where plastic pollution was not an explicit threat or if the study was a review that lacked original data. Entanglement in active fishing gear (i.e. bycatch) was not considered, but entanglement in derelict gear was. Data were collated based on the lowest taxonomic group, interaction type (i.e. entanglement and/or ingestion), individual effect (i.e. lethal or sub-lethal), location of interaction (i.e. ocean region), number of individuals impacted, and whether the study provided sufficient evidence (e.g. mortality levels/rates, affected life stage) to demonstrate population-level impacts, or the lack thereof at the time of the study. Effects were classified as “lethal” if an animal died directly from plastic, and all other effects were considered “sub-lethal”.

## Summary of review: Current state of knowledge

In total, 253 published studies documented plastic pollution in marine megafauna, either by entanglement (*n*=54), ingestion (*n*=180), or both (*n*=19) (Dataset S1). We found that the cumulative number of studies on entanglement in and ingestion of plastic pollution published from 1969–2018 increased in all three taxa (Figure 2). Of all published studies, 40 reported lethal (*n*= 9, entanglement; *n*=26, ingestion; *n*=5, entanglement and ingestion) and 77 reported sub-lethal (*n*=35, entanglement; *n*=37, ingestion; *n* =5 entanglement and ingestion) effects of plastic pollution. Lethal and sub-lethal effects included gastrointestinal tract damage (*n*=44), reduced mobility at sea or on land (*n*=24), physical injury (*n*=18), starvation (*n*=16), drowning (*n*=12), nutrient dilution (*n*=7), constriction (*n*=6), malnutrition (*n*=6), physiological stress

(*n*=2), and exposure to toxicants (*n*=1). Most research was conducted in the Atlantic (54%) and Pacific (32%) Ocean regions (Figure 2, Dataset S1).

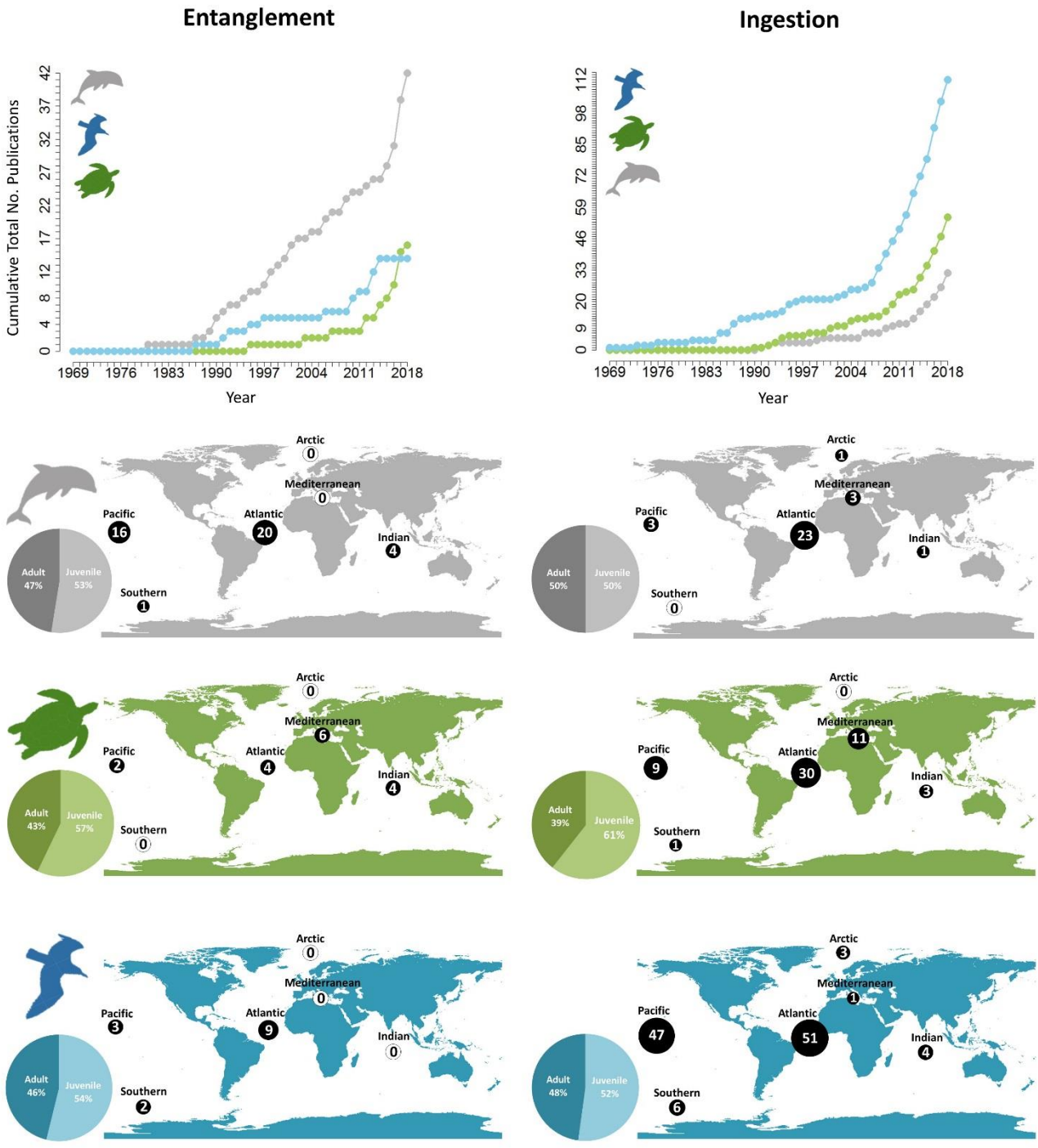


**Figure 2.** The number of peer-reviewed studies investigating plastic pollution and seabirds, marine mammals, and sea turtles per year (published between 1969 and 2018), per ocean basin and proportion per age-class (adult and juvenile).

All seven sea turtle species, 53% of the 346 seabird species (BirdLife International 2012), and 42% of the 128 marine mammal species (Society for Marine Mammalogy 2016) had documented interactions with plastic pollution. Ingestion studies of seabirds comprised 45% of all published entanglement and ingestion studies, combined (Figure 2; Dataset S1). The species with the highest number of publications from either entanglement and/or ingestion included loggerhead turtles (*Caretta caretta*; n=33) and northern fulmars (*Fulmarus glacialis*; n=25), which comprised 10 and 13% of all papers, respectively (Dataset S1).

Loggerhead turtles (14%), harbour seals (*Phoca vitulina*; 11%), California sea lions (*Zalophus califomiaous*; 10%), olive ridley turtles (*Lepidochelys olivacea*; 8%), and northern gannets (*Morus bassanus*; 8%) were most frequently represented in the entanglement studies, whereas loggerhead turtles (14%), green turtles (*Chelonia mydas*; 14%), northern fulmars (12%), and great shearwaters (*Ardenna gravis*; 8%), were most frequently represented in the ingestion studies (Dataset S1). Harbour seals (19%), loggerhead and olive ridley turtles (63% and 38%, respectively), and northern gannets (38%) had the highest number of entanglement publications for marine mammals, sea turtles, and seabirds, respectively (Dataset S1). By contrast, sperm whales (*Physeter microcephalus*; 10%), loggerhead turtles (51%), and northern fulmars (21%) had the highest number of ingestion publications for marine mammals, turtles, and seabirds, respectively (Dataset S1).

To date, 32% (*n*=80) of studies discussed potential population-level consequences of plastic pollution for at least one species evaluated in their study (Figure 3).

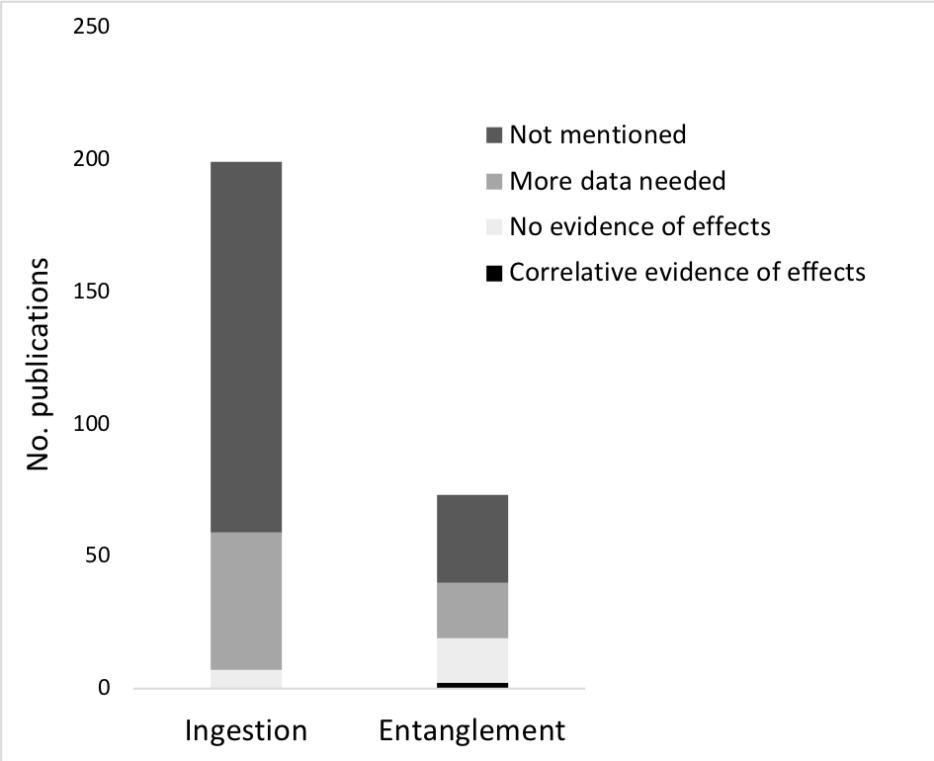


**Figure 3.** Number of published, peer-reviewed studies that evaluated or discussed population-level effects of plastic pollution on seabirds, marine mammals, and sea turtles between 1969 and 2018.

Twenty-four studies suggested the possibility of population-level effects without providing their own evidence (*n*=7, entanglement; *n*=17, ingestion), while 78 studies concluded that more information is needed to assess effects on populations (n=19, entanglement; n=40, ingestion; n = 19, ingestion and entanglement) (Figure 3; Dataset S1). Overall, we were unable to find a study that provided direct evidence of population-level effects, although two studies found correlative or inferred effects (Figure 3 and Table 1; Dataset S1). By contrast, we found 21 studies that were unable to demonstrate effects at the time of study (Figure 3 and Table 1; Dataset S1). Keeping in mind the apparent lack of research on or evidence of population-level effects of plastic pollution on marine megafauna, we evaluate results of our literature review by effect types and taxonomic group.

**Table 1.** Summary of peer-reviewed studies published between 1969 and 2018 that evaluated potential population-level consequences of plastic pollution based on reported lethal or sub-lethal effects.

| Taxa | Species | Threat; *N* entangled/ ingested; Study period | Lethal or sub-lethal effect | Age class | *Evidence of population-level effect | Key findings | Reference |
|---|---|---|---|---|---|---|---|
| Marine Mammal (Cetacean) | Franciscana dolphin (*Pontoporia blainvillei*); Guiana dolphin (*Sotalia guianensis*) | Ingestion; 15 (14 Franciscana, 1 Guiana); Unspecified | 166 dead animals, but plastic ingestion not the reported cause of death. | Unspecified | Unable to demonstrate | Impacts of debris ingestion appear to be minimal considering no lethal or sublethal effects were reported. | Madeira di Beneditto and Arruda Ramos (2014) |
| Marine Mammal (Cetacean) | Harbour porpoise (*Phocoena phocoena*); harbour seals (*Phoca vitulina*); grey seal | Ingestion and entanglement; 31; 1990-2014 | 6,587 carcasses found, of which 1622 allowed for necropsy; 14 cases of entanglement and 17 of ingestion reported. | Juvenile; Adult | Unable to demonstrate | Despite high standings, few animals interacted with plastic; these rates are unlikely to cause population-level effects. | Unger et al. (2017) |

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| | (*Halichoerus grypus*) | | | | | | |
| Seabird | Short-tailed Shearwater (*Puffinus tenuirostris*) | Ingestion; 164; 2012 | 171 birds necropsied, but plastic did not affect body condition of 164 that exhibited ingestion. | Chick | Unable to demonstrate | Plastic ingestion does not appear to affect the birds' body condition, despite high ingestion. | Cousin et al. (2015) |
| Seabird | Wandering albatross (*Diomedea exulans*); black-browed albatross (*Thalassarche melanophris*); grey-headed albatross (*Thalassarche chrysostoma*) | Ingestion; 52 (46 wandering, 3 black-browed, 3 grey-headed); 1993-2009 | Hook/line ingestion; mortality unspecified. | Adult | Unable to demonstrate | Wandering albatross populations have declined sharply, but no evidence links this decline to plastic ingestion; other populations show no evidence of decline due to plastic ingestion. | Phillips et al. (2010) |
| Seabird | Southern giant petrel | Ingestion; 193; 2001-2004 | 193 animals ingested plastic, | Chick | Unable to demonstrate | Population has stabilized; no evidence of population-level effects. | Copello et al. (2008) |

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| | (*Macronectes giganteus*) | | none of which were attributed to plastic. | | | | |
| Seabird | Waved albatross (*Phoebastriair rorata*) | Ingestion; 6; 1999-2007 | 43 dead animals, 6 of which ingested plastic; mortality not attributed to ingestion. | Chick; Adult | Unable to demonstrate | Population is declining, but unlikely because of plastic pollution. | Anderson et al. (2008) |
| Turtle | Loggerhead turtle (*Caretta caretta*) | Ingestion; 121; 1995-2016 | Despite the high occurrence of debris in loggerheads observed in the study (1414 plastic items collected in 121 of the 155 turtles analyzed), there was little evidence that debris caused impactions, obstructions, or perforations in the gut; the small amount of debris was also suggestive | Juvenile; Adult | Unable to demonstrate | The amounts of ingestion by juvenile loggerheads that inhabit the western Mediterranean are low and does not appear to pose a significant threat to the survival of their populations in the region. | Domènech et al. (2019) |

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| | | | of little dietary dilution. | | | | |
| Marine Mammal (Pinniped) | Fur seal (*Arctocephalus spp.*); south elephant seal (*Mirounga leonina*) | Entanglement; 106 (101 fur seals, 5 elephant seals); 1991-2001 | None documented | Unspecified | Unable to demonstrate | 0.24% of population entangled; no clear effect on population. | Hofmeyr et al. (2002) |
| Marine Mammal (Pinniped) | Pacific harbour seal (*Phoca vitulina*) | Entanglement; 11; 2001-2005 | Unspecified | Unspecified | Unable to demonstrate | Entanglement unlikely to affect study population. | Moore et al. (2007) |
| Marine Mammal (Pinniped) | Australian fur seal (*Arctocephalus pusillus*) | Entanglement; 74; 1997-2012 | None documented | All age classes | Unable to demonstrate | Population is currently increasing; entanglement unlikely to affect population. | Lawson et al. (2015) |
| Marine Mammal (Pinniped) | Antarctic fur seal (*Arctocephalus gazella*) | Entanglement; 208; 1988-1989 | None documented | All age classes | Unable to demonstrate | Current rate of entanglement (0.4%), most of which are juvenile males, unlikely to impact population. | Arnould and Croxall (1995) |

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| Marine Mammal (Pinniped) | California sea lion (*Zalophus californianus*) | Entanglement; 237; 1991-1995 | None documented | All age classes | Unable to demonstrate | The current entanglement rate (0.49%) is unlikely to cause population-level impacts. | Zavala Gonzalez and Mellink (1997) |
| Marine Mammal (Pinniped) | Antarctic fur seal (*Arctocephalus gazella*) | Entanglement; 1,033; 1989-2013 | One death reported due to entanglement. | All age classes | Unable to demonstrate | Rates of entanglement are low (0.016%) and involve mostly juvenile males; entanglement unlikely to affect population. | Waluda and Staniland (2013) |
| Marine Mammal (Pinniped) | Australian fur seal (*Arctocephalus pusillus doriferus*) | Entanglement; 359; 1997-2013 | None documented | All age classes | Unable to demonstrate | Population has been recovering since protection in 1975. | McIntosh et al. (2015) |
| Marine Mammal (Pinniped) | Australian fur seal (*Arctocephalus pusillus doriferus*) | Entanglement; 106; 1996-2002 | Entanglement rates range from .024% to .059% per season; 1 death attributed to entanglement. | All age classes | Unable to demonstrate | Entanglement rates are considered negligible and unlikely to impact population. | Hofmeyr et al. (2006) |
| Marine Mammal (Pinniped) | Australian sea lion (*Neophoca* | Entanglement; | High rates of entanglement (45% in fur seals and 74% | All age classes | Unable to demonstrate | Despite high occurrence of entanglement, fur seal populations increasing by | Page et al. (2004) |

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| | *cinerea*)*;* New Zealand fur seal (*Arctocephalus forsteri*) | 126 (35 sea lions, 91 fur seals); 1989-2002 | in sea lions), only 5 of which were killed (fur seals). | | | 16%; stable sea lion populations, although entanglement-related mortality is likely slowing their recovery. | |
| Marine Mammal (Pinniped) | California sea lion (*Zalophus californianus*); Steller sea lion (*Eumetopias jubatus*) | Entanglement; 914; 1976-1998 | 914 pinnipeds reported entangled. | All age classes | Unable to demonstrate | Population of California sea lions increasing, while entanglement rates were not significant for Stellar sea lions. | Hanni and Pyle (2000) |
| Marine Mammal (Pinniped) | New Zealand fur seal (*Arctocephalus forsteri*) | Entanglement; 185; 1995-2005 | 185 seals reported entangled over past ten years, with average of 19 ± 2; 4 deaths attributed to entanglement. | All age classes | Unable to demonstrate | Low mortality rates due to mortality; population appears to be increasing. | Boren et al. (2006) |
| Marine Mammal (Pinniped) | California sea lion (*Zalophus californianus*) | Entanglement; 157; 2001-2005 | Unspecified | Unspecified | Unable to demonstrate | Entanglement rate for California sea lions (0.03%) is unlikely to impact population. | Moore et al. (2009) |

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| Marine Mammal (Pinniped) | Northern fur seals (*Caiiorhinus urslnus*) | Entanglement; ;Unspecified; 1960-1895 | .0.4% entanglement rate in 1985, at least two orders of magnitude greater than in the 1940s. | Juvenile; Adult | Correlative | Changes in pup numbers and unexpected mortality in juveniles provide correlative evidence for population decline. | Fowler (1987) |
| Marine Mammal (Cetacean) | Northern right whale (*Eubalaena glacialis*) | Entanglement; 21; 1979-2009 | Entanglement responsible for increased energy cost and drag, impeded foraging efficiency. | Juvenile; Adult | Inferred from sub-lethal impacts | In affected population, chronic entanglement causes greater energy costs, impeding reproductive investment and blubber thickness. | Van der Hoop et al. (2017) |
| Turtle | Olive ridley turtle (*Lepidochelys olivacea*) | Entanglement; 18; 1996-2011 | 18 entangled turtles, 2 of which were reported dead. | Juvenile | Unable to demonstrate | 18 entanglement events across 15 years unlikely to affect population. | Santos et al. (2012) |
| Seabird | Northern Gannet (*Morus bassanus*) | Entanglement; 525; 2005-2010 | 195 of 525 entangled animals died; 190 of which were attributed to entanglement. | Juvenile | Unable to demonstrate | Presence of plastic in population is concerning, but currently not impacting population. | Votier et al. (2011) |

*Indicates evidence or lack of evidence for population-level impacts of plastic pollution only at time of study.

## Taxonomic and spatial extent of lethal and sub-lethal effects

### *Entanglement*

*Marine mammals*

A total of 42 studies was found linking entanglement in plastic with marine mammals, of which 30 reported either lethal (10%) or sub-lethal (62%) effects (Dataset S1). Pinnipeds (55%) and cetaceans (38%) comprised the majority of studies by taxon (Dataset S1). In terms of lifestage, immature marine mammals were most commonly reported in entanglement studies (74% of studies; Figure 2, Dataset S1). The Pacific and Atlantic Ocean regions accounted for the majority of entanglement studies (Figure 2; Dataset S1).

*Sea turtles*

A total of 16 studies was found linking entanglement in plastic with sea turtles, of which 14 reported either lethal (19%) or sub-lethal (63%) effects (Dataset S1). Loggerhead (63%) and olive ridley turtles (38%) were the most frequently reported species in entanglement publications (Dataset S1). Immature turtles accounted for 75% of all entanglement studies (Figure 2; Dataset S1). The Mediterranean, Atlantic, and Indian Ocean regions accounted for the majority of entanglement studies (Figure 2; Dataset S1).

*Seabirds*

A total of 16 studies was found linking entanglement in plastic with seabirds, of which 10 reported either lethal (44%) or sub-lethal (25%) effects (Dataset S1). Northern gannets (38%) were the most frequently reported species in entanglement publications (Dataset S1). Adult seabirds accounted for 50% of all entanglement studies (Figure 2; Dataset S1). The Atlantic and Pacific Ocean regions comprised the majority of entanglement publications (Figure 2; Dataset S1).

### *Ingestion*

*Marine mammals*

A total of 30 studies reported ingestion of plastic by marine mammals, of which 11 reported either lethal (37%) or sub-lethal (37%) effects (Dataset S1). Cetaceans (70%) comprised the majority of studies by taxon (Dataset S1). In terms of lifestage, adult and immature marine mammals were equally reported in ingestion studies (57% of studies; Figure 2; Dataset S1). The Atlantic and North Sea Ocean regions accounted for the majority of entanglement studies (Figure 2; Dataset S1).

*Sea turtles*

A total of 55 studies was found linking ingestion of plastic by sea turtles, of which 35 reported either lethal (18%) or sub-lethal (38%) effects (Dataset S1). Loggerhead (51%) and green turtles (49%) were the most frequently reported species in ingestion publications (Dataset S1). Immature turtles accounted for 73% of all turtle entanglement studies (Figure 2; Dataset S1). The Atlantic Ocean region accounted for the majority of ingestion studies (Figure 2; Dataset S1).

*Seabirds*

A total of 112 studies was found linking ingestion of plastic by seabirds, of which 16 reported either lethal (8%) or sub-lethal (5%) effects (Dataset S1). Northern fulmar (21%) and great shearwaters (13%) were the most frequently reported species in ingestion publications (Dataset S1). Adult seabirds accounted for 52% of all ingestion studies (Figure 2; Dataset S1). The Atlantic and Pacific Ocean regions comprised the majority of entanglement publications (Figure 2; Dataset S1).

## Overview and implications of marine megafauna interactions with plastic pollution

### *Entanglement and other external effects*

Marine megafauna can become entangled in plastic pollution including fibrous material, line, rope, packing bands, netting, and other packaging material. Animals

may be attracted to plastic material in several ways, including: 1) curiosity or naivety (especially in immature animals); 2) to use as a resting platform or for shelter; or 3) to seek prey that is either entangled or attracted to the material (Matsuoka et al., 2005; Gregory, 2009; Jensen et al., 2013; Duncan et al. 2017). If entanglement does not lead to immediate mortality (e.g. from drowning), stress may cause acute and chronic effects on important behavioral and physiological processes such as predator avoidance, foraging, energy assimilation, migration, mating, nesting, and care of offspring. Ghost or derelict fishing gear (i.e. gear that is abandoned, lost, or deliberately discarded) can continue “fishing” and entangle marine megafauna for long periods of time (Baulch and Perry 2014; Matsuoka et al. 2005; Gilardi et al. 2010; Wilcox et al. 2014). Recent estimates suggest that 6.4 million tons of fishing gear is lost annually and is increasing worldwide (Macfayden et al. 2009; Wilcox et al. 2014). When compared to other plastic items, derelict fishing gear (e.g., nets, pots, traps, lines, buoys) is believed to cause the greatest impact to marine megafauna (Wilcox et al. 2016; Duncan et al. 2017).

*Physical injury and illness*

Entanglement in plastic pollution can lead to physical injuries that include lacerations, constriction, severe sclerosis, loss of limbs, and difficulty breathing if the airway becomes restricted (Wegner and Cartamil 2012; Snoddy et al. 2009; Vegter et al. 2014). The animal may starve, drown, or be unable to escape predators or vessels if the entangled material hampers movement (Gregory 2009; Barreiros and Raykov, 2014; Vegter et al., 2014, Nelms et al. 2015). For example, cetaceans entangled in plastic ropes, lines, and floats may develop systemic infections and chronic debilitation from extensive tissue damage (Cassoff et al. 2011), and pinnipeds have been known to insert their heads through plastic packing bands, which can eventually lead to severed blood vessels (Fowler, 1987). Entanglement of seabirds, both at sea and at terrestrial breeding sites, may reduce their flying and foraging efficiency (Derraik 2002).

*Physiological stress*

Entanglement in plastic pollution can result in severe physiological stress, inhibiting

diving and resulting in increased hydrodynamic drag (Ceccarelli 2009; Macfayden et al. 2009; Gilardi et al. 2010; Van de Hoop et al. 2013b; Wilcox et al. 2014). For example, an entangled North Atlantic right whale (*Eubalaena glacialis*) incurred an increase in average locomotory power requirements of 70.5% when entangled in plastic rope (Van de Hoop et al. 2013b), while energy requirements for a California sea lion entangled in plastic netting increased four-fold (Feldkamp 1985). In laboratory experiments, entangled fur seals reduced swimming time by 75%, increased resting by 138%, and increased their mean energy expenditure by 112% (Feldkamp et al. 1989).

Several studies have shown that sea turtles entangled in fishing gear – an experience that induces similar physiological responses to entanglement in derelict gear – require additional time to rest and recover at the surface to replenish on-board oxygen stores consumed while involuntarily submerged (Gregory et al. 1996; Stabenau and Vietti 2003; Snoddy and Williard 2009; Snoddy et al. 2009). Blood samples from wild Kemp's ridley turtles (*Lepidochelys kempii*) entangled in nets demonstrated that submergence significantly influenced the time course of recovery of blood homeostasis (Hoopes et al. 2000). Wild green turtles captured (and submerged) in gillnets have exhibited blood lactate levels indicative of severe metabolic acidosis and have shown substantial changes in blood ion levels (sodium [Na+], chloride [Cl–], and potassium [K+]) (Snoddy et al. 2009). Cardiac muscle damage may also occur from overexertion during entanglement (Snoddy et al. 2009). Greater entanglement durations in green and Kemp's ridley turtles have resulted in decreased health, shown by physical examination, and a significant increase in blood lactate, lactate dehydrogenase and creatine phosphokinase enzymes, phosphorus, and glucose levels (Snoddy et al. 2009). Similarly, a threefold increase in plasma corticosterone – a hormone representative of stress (Gregory et al. 1996) – and a decrease in blood pH (Harms et al. 2003) were reported in loggerhead turtles submerged for only 30 minutes that were captured in trawl, entanglement, and pound nets. Such dive durations are not uncommon among freely diving juvenile and adult sea turtles while performing natural behavior; thus, these findings indicate physiological derangement caused by stress of entanglement, not necessarily physiological limitations on aerobic dive activity. Upon release from

entanglement (and submergence), green and Kemp's ridley turtles spent extended periods of time recovering at the surface, potentially increasing their vulnerability to predation and anthropogenic threats, such as vessel strikes (Snoddy et al. 2009; Snoddy and Williard 2010). Despite an apparent lack of similar studies for seabirds, these animals are also likely to experience some form of physiological stress from submersion due to entanglement.

*Reduced mobility*

Plastic pollution may also impede, obstruct, or entrap marine megafauna that rely on terrestrial environments for resting or reproduction (e.g. seabirds, pinnipeds, and sea turtles). Plastic material has been known to affect adult and nestling seabirds, entangling their legs, feet, bill, and wings (Tasker et al. 2000; Votier et al. 2011; Bond et al. 2012). Synthetic materials present on sea turtle nesting rookeries can block nesting attempts or impede hatchlings. For example, on a Mediterranean beach in Turkey, plastic objects impeded hatchling sea turtles' attempt to reach the sea, potentially making them more susceptible to predation and decreased energy reserves required for the frenzy swim upon entering the water (Triessnig et al. 2012). On Elliot Key, Florida, USA, extensive derelict fishing gear and other beach cast debris, consisting largely of plastic, was believed to be preventing nesting by sea turtles. Potential evidence of this effect came following removal of the items (3.4 tons), when nesting by loggerhead and green turtles resumed (Coastal Cleanup Corporation, Suzy Pappas pers. comm.).

**Ingestion**

It is believed that marine megafauna may ingest plastic: 1) by mistaking the item for food (Gregory, 2009; Hoarau et al., 2014; Schuyler et al, 2012; Schuyler et al, 2014b); 2) accidentally through non-selective feeding strategies, such as filter feeding (Fossi et al. 2014) or if otherwise mixed with natural food items (Di Beneditto and Awabdi, 2014); 3) if the item is attached or covered with natural prey (Frick et al. 2009); or 4) via trophic transfer from contaminated prey (Nelms et al 2018).

*Gastrointestinal tract damage*

Ingested plastic objects may damage the gastrointestinal tract (GIT) of marine megafauna by causing ulcerations, perforations, lesions, and obstructions (Derraik 2002; Jacobsen et al. 2010; Brandão et al. 2011; Awabdi et al. 2013; Di Bello et al., 2013; Di Beneditto & Awabdi 2014; Nelms et al. 2015). Gastrointestinal ulcerations or perforations and laceration of the larynx from ingesting plastic have been documented in marine mammals, sea turtles, and seabirds, and can result in chronic infection, peritonitis, gastrointestinal motility issues, septicemia, and mortality (Day et al. 1985, McCauley & Bjorndal 1999, Levy et al. 2009; Guebert-Bartholo et al. 2011). Impaction or blockage of the gastrointestinal tract caused by plastic ingestion can inhibit digestion and cause pain, bloating, necrosis, hardened fecal matter, mechanical abrasion or blockage of absorptive surfaces in the digestive tract, and blockage of the cloaca which can prevent egg laying (Mader 2006; Guebert-Bartholo et al. 2011; Awabdi et al. 2013; Di Beneditto and Awabdi 2014). Seabirds that ingest high levels of plastic may exhibit slower growth rates and earlier mortality (Pierce et al. 2004), while gut compactions and minor ulcerations caused by plastic ingestion in seabirds may result in reduced disease resistance and post-fledging survival (Fry et al. 1987).

*Dietary dilution*

Dietary dilution can occur when ingestion of plastic limits nutrient or water absorption. The presence of inorganic and space-occupying, non-food material within the GIT can cause a false sense of satiation, leading to a reduced desire to feed (McCauley and Bjorndal 1999). Nutrient dilution is known to affect both juvenile and adult animals (Day et al. 1985; Sievert & Sileo 1993; Bjorndal et al. 1994; McCauley & Bjorndal 1999). Although sub-lethal, dietary dilution may lead to malnutrition, reduced energy, and eventual mortality. Loggerhead turtle hatchlings fed a 50% diluted diet with inert matter (fumed silica) displayed significantly lower energy and nitrogen intake than hatchlings fed a 10% diluted diet, indicating that dietary dilution may decrease energy assimilation and allocation to somatic growth, which could reduce energy reserves and survivorship (McCauley & Bjorndal 1999). Similarly, dietary dilution may dehydrate seabird chicks with already reduced fat

reserves (Auman et al. 1997). Growth rates for Laysan albatross (*Phoebastria immutabilis*) that had ingested high volumes of plastic were significantly lower than for chicks that had ingested low volumes of plastic (Sievert & Sileo 1993). Decreased body condition (reduced fledging weight), which can result from dietary dilution, has been found to decrease survival of juvenile seabirds (Braasch et al. 2009; Morrison et al. 2009).

*Exposure to contaminants associated with plastic pollution*

Plastic can adsorp and concentrate chemical contaminants, such as persistent organic pollutants (POPs), from the marine environment (Teuten et al. 2009). These toxic compounds can be harmful because they are inherently stable, persist for a long time, and can accumulate in adipose (fatty) tissues following ingestion (D'Ilio et al. 2011). Many common polymers, such as polyethylene, have high sorptive capacities for toxicants due to their polymeric chain structure and enhanced surface area (Rochman et al. 2013). This capacity increases with degradation and a corresponding increase in surface area, which leads to the plastic becoming more hazardous the longer it remains in the marine environment (Andrady 2011). In addition to the adsorption of existing marine contaminants to their surfaces, plastic often contains toxic additives, monomers, and chemical byproducts as well as plasticizers, such as phthalates and Bisphenol A (BPA), added during manufacturing (Teuten et al. 2009; Lithner et al. 2011).

POPs in marine megafauna tissues have been linked to plastic ingestion. Colabuono et al. (2010) found Polychlorinated biphenyls (PCBs) and organochlorine pesticides (OCPs) in plastic pellets and fragments ingested by Procellariiforme seabirds in Southern Brazil, while Tanaka et al. (2013) reported that short-tailed shearwaters (*Puffinus tenuirostris*) found with plastic in their stomachs in the North Pacific had polybrominated diphenyl ethers (PBDEs) in their abdominal adipose, which was also found in the same pieces of plastic. Similarly, lower chlorinated compounds were found to have transferred to short-tailed shearwaters as a result of ingesting contaminated plastic (Yamashita et al. 2011). At Midway atoll, PCBs, polychlorinated dibenzo-p-dioxins, naphthalenes, and furans have been found in adult Laysan albatrosses (Jones et al. 1996), while PCBs were found to have transferred from

contaminated plastic to streaked shearwater chicks in a feeding experiment (Teuten et al. 2009).

In addition to potential toxicity contamination via ingestion, seabirds that nest on top of plastic material may absorb contaminants through their skin (Verlis et al. 2014), which could affect sexual development and potentially disrupt the endocrine system, resulting in reproductive difficulties and cancers (vom Saal et al. 2007; Talsness et al. 2009). Prior research has found that transfer of chemicals that commonly occur in plastic (e.g. BPA) can occur through the skin (Geens et al. 2011; Zalko et al. 2011).

## **Knowledge gaps and research priorities**

*Assessment of available literature*

In many marine megafauna species, particularly cetaceans and turtles, effects of plastic pollution may occur several to greater than hundreds of miles offshore, and subsequent mortality may be difficult, if not impossible, to assess (Gregory 2009). Thus, mortality is likely to be grossly under-reported. The comparatively large number of studies that reported lethal effects in pinnipeds suggests that, they are either disproportionately impacted by entanglement, or that entanglement in plastic, and subsequent mortality, is more visible and thus easier to document given their close association with terrestrial habitats. It is also likely that pinniped populations have relatively smaller home ranges relative to other taxa. Although sea turtles are also associated with terrestrial environments (i.e. nesting females), they tend to have larger ranges than pinnipeds and are not closely linked to terrestrial environments for the vast majority of their lives. Turtles that suffer serious injuries and subsequent mortality from plastic pollution may be more likely to die in open water, especially small juveniles.

Among marine mammals, little information exists on plastic pollution impacts to sirenians and baleen whales. Given sirenians' affinity for nearshore and inland environments worldwide, it is surprising that only two studies ($n$=1, entanglement; $n$=1, ingestion) have documented their interaction with plastic. This lack of information could be an indicator that plastic pollution may not pose a serious threat to these animals, that they interact with plastic differently than other megafauna, or

that such observations are not generally reported in peer-reviewed literature because they are often incidental and made by non-scientists. While baleen whales are known to interact with fisheries (Read et al. 2006), differentiating between interactions with active fisheries (i.e. bycatch) and ghost fishing gear (i.e. plastic pollution) is challenging, which may explain the small number of baleen whales that were definitively linked to entanglement in plastic.

Virtually no studies have been carried out in the Arctic and Southern Ocean, which is also surprising given that many scientists are working in these regions and they represent global hotspots for several marine megafauna species. The lack of studies may indicate that animals from these regions are not as exposed to plastic pollution as those inhabiting coastal regions more populated by humans, and thus more likely to receive terrestrial inputs. Alternatively, these regions may pose logistic issues associated with data collection (e.g. working from a boat with limited or no access to the shore).

*Individuals, species, and habitats affected*

Although a variety of marine megafauna are known to suffer detrimental effects caused by plastic pollution, the list of species is incomplete (but growing), as is the catalog of effects on individual animals (Vegter et al. 2014; Gall and Thompson 2015). A more representative dataset of these effects, with spatial and temporal variation represented, will be necessary to better discern patterns and trends. Where differential effects on species have been examined (Di Beneditto & Awabdi 2014), patterns are apparent, but effects from habitats that differ by species remain unknown. Clearly, some marine habitats bear an especially high plastic pollution load, such as remote islands within oceanic current fields (e.g. Midway Atoll), while some populations may be more resilient to impacts (e.g. certain pinnipeds and large whales). Spatial hotspots in plastic hazards have shown to be associated with dynamic oceanographic and geographic features such as frontal zones (Carman et al. 2014; Witherington 2002; Witherington et al. 2012) as well as proximity to human population centers (Browne et al. 2010; Carman et al. 2014). Although understanding the impacts of marine plastic does not require a complete dataset on

the spatio-temporal intersection of plastic and marine megafauna by species and population, it will require sufficient representative data to model these effects.

Some marine megafauna can apparently overcome certain plastic pollution impacts, although the mechanisms are not fully understood. For example, photographic studies of humpback whales entangled in ghost (or active) gear in Alaska and the Gulf of Maine, USA, revealed that the majority of animals (52-78%) had been non-lethally entangled, suggesting that animals were able to free themselves (Neilson et al. 2009). In the Gulf of Maine, 48-65% of humpbacks were entangled between 1997 and 2002, of which 8-25% were estimated to entangle annually (Robbins and Mattila, 2004). Similarly, approximately 37% of 371 leatherback turtles autopsied from 1968 – 2007 had plastic in their GI tract (Mrosovsky et al. 2009), yet of those, only 12 (8.7%) appeared to die from it. By contrast, documented entanglement rates for grey seals (*Halichoerus grypus*) from photo ID techniques in southwest England from 2004 to 2008 revealed that 64% of entanglement events resulted in serious injuries, with significantly lower recapture rates of entangled seals, suggesting an elevated post-release mortality rate (Allen et al. 2012). More information is needed to understand why some species or populations appear to be more resilient to impacts than others.

*Population-level assessments*

In general, potential population-level consequences of plastic pollution in marine megafauna have infrequently been discussed, and have yet to quantified (Figure 3; Table 1). Despite reviewing more than 100 published studies of lethal and sub-lethal effects, we were unable to find population-level assessments of changes in vital rates (e.g., survivorship, fecundity, somatic growth, life-stage duration) or abundance trends resulting from plastic hazards exposure (Figure 3; Table 1). Two studies, however, reported correlative and inferred evidence of effects, both of which resulted from entanglement (Figure 3; Table 1).

Although the harm from plastic to marine megafauna is widespread, it is unclear if or how many populations are significantly affected, and which populations show the greatest effects. A critical question is whether the lack of information represents a lack of actual effects or a lack of investigation? Effects could be widespread and

important for populations, but are not yet known and quantified. As plastic pollution continues to increase worldwide, its potential to cause population effects may increase or become easier to assess. Conversely, plastic pollution may not present a major conservation threat to marine megafauna at current levels.

Establishing population baselines in many megafauna populations presents challenges due to their extensive migrations and large ranges. Thus, estimating trends can be difficult in species where we lack sufficient population information. A crucial step will be to estimate the proportion of individuals in a population that are killed by plastic exposure in relation to their population size as well as the mortality they incur from other anthropogenic impacts (Browne et al. 2015). Population models can help elucidate potential impacts by incorporating a suite of metrics including relative contribution of a given lifestage (e.g. mature females), the size and growth rate of a population, mortality rates of different lifestages, and reproductive parameters (Browne et al. 2015).

*Multidimensional effects: plastic as an exposure route for associated chemical pollutants*

The acute, coarse-scale effects from plastic pollutants (e.g., entanglement, gut impaction) are more easily demonstrated than chronic effects on a finer scale. Where investigated, microplastics appear to be ubiquitous in marine megafauna (Duncan et al. 2018; Lusher et al. 2018; Nelms et al. 2019). However, gaps still remain in our understanding of plastic ingestion, particularly microplastics and their potential to transfer and persist up marine food webs to marine megafauna or to be ingested directly by them. Otoliths of night-feeding pelagic fish (*Electrona subaspera)* consumed by Hooker's sea lions (*Phocarctos hookeri*) have been found alongside small plastic fragments (~ 1 mm diameter) in their scat that presumably had been originally consumed by the fish (McMahon et al., 1999). Similarly, scat from fur seals at Macquarie Island, Australia, contained small plastic particles (< 10 mm) believed to similarly accumulate through food-web transfer from the same myctophid fish (Eriksson & Burton 2003). Ingestion of microplastics by animals at the base and middle of the food chain, such as zooplankton and epipelagic zooplanktivorous fish,

could also facilitate trophic transfer to marine megafauna (Boerger et al. 2010; Davison et al. 2011; Eriksen et al. 2014; Nelms et al. 2018).

The large surface area to volume ratio of microplastics (particles ≤ 5mm) means that they are susceptible to the adsorption of hydrophobic contaminants from seawater, and research suggests that many more species than previously thought ingest them (Browne et al. 2008; Cole et al. 2011). The recent increase in products with antimicrobial silver nanoparticles (Blaser et al. 2008) and endocrine-disrupting brominated flame retardants (Hammer et al. 2012) may mark a trend in a proliferation of chemically altered synthetic products. Low-trophic organisms, such as fish and lugworms, may also transfer toxicants up the food web to marine megafauna by ingesting contaminated plastic (Teuten et al., 2009; Rochman et al. 2013). Future research should assess the levels and effects of both microplastic and contaminants in animals of lower trophic levels including the role that ingestion may play in biomagnifying toxic chemicals common in plastic pollution up the food chain to marine megafauna.

*Exposure risk of plastic hazards*

Risk associated with marine plastic hazards is the measure of probability that harm will occur given a specific level of exposure, with exposure related to dose (e.g. plastic burden) and time. In the simplest terms, risk is related to a function of hazard multiplied by dose and time. Thus, plastic that poses only a small hazard, but is frequent or available for extended periods of time, may pose a risk similar to that of greater hazards with more limited exposure. Analyses like these for marine megafauna are lacking due to the paucity of data on each element of the risk equation. Hazards of marine plastic are incompletely described, either in their immediate effects (entanglement, physical effects in the gut, toxicants from manufacturers) or in their eventual effects following UV exposure, fragmentation, adsorption of environmental toxicants, and additional chemicals shed during digestion. Stressor-response profiles have not been developed for this suite of plastic hazards. One notable exception, however, used logistic regression to estimate that sea turtles with a curved carapace length of 43.5 cm had a 50% probability of mortality after ingesting 14 plastic items, with a 22% probability of

death from ingesting a single item that reached 100% with 226 items (Wilcox et al. 2018). This study serves as an important catalyst for future research to assess the extent to which concentrations of plastic may result in physiological and anatomical impairment that can lead to death.

Methodologies used to assess risk exposure need to be carefully considered. Several ingestion studie have excluded non-detects which can lead to substantial overestimations of mean ingestion amounts (Lynch 2018). Researchers are now beginning to call for studies to publish both positive and negative results to better understand the overall impacts of plastic pollution (Nelms et al. 2015; Lynch et al. 2018). Moreover, most sea turtle and seabird plastic ingestion studies have used frequency of occurrence (%FO) to assess ingestion; however, %FO does not depict the amount of debris that was actually ingested by the animal, which limits its usefulness and can substantially bias results in terms of actual risk (Lynch 2018). Where possible, Lynch (2018) recommends that researchers measure plastic ingestion by debris mass per turtle mass (g/kg) in order to better identify at-risk populations.

Differences in plastic collection techniques from dead (e.g. causes of death for necropsied animals) or alive (e.g. esophagus lavage or feces) animals also make it difficult to draw meaningful comparisons within and amongst studies (Casale et al. 2016; Lynch 2018; Rodríguez et al. 2018; Nelms et al. 2019), while strandings are not indicative of actual mortality rates in a given population (Epperley et al. 1996; Williams et al. 2011; Casale et al. 2016). It is recommended that future studies either only include animals that died immediately and were presumably healthy and feeding normally, such as seabird fledglings grounded by lights, or strive to include these animals as a comparison (Casale et al. 2016; Rodríguez et al. 2018).

*Laboratory and field research*

A surprisingly small amount of plastic (i.e. 0.5 g or one-tenth of a typical plastic bag) can block the digestive tract in juvenile green turtles (Santos et al. 2015), yet as much as 75 g (149 plastic items) can accumulate and remain in the gut of sea turtles without causing apparent damage (Hoarau et al. 2014). In one case, plastic remained in the gut of an apparently healthy captive loggerhead turtle for four

months (Lutz 1990). Clearly, more experimental research is needed to understand the effects of ingesting plastic.

Controlled studies are needed to assess post-entanglement and post-ingestion fate in marine megafauna. Captive animals that could serve as surrogates for endangered species could be used to better understand potential sub-lethal and lethal responses, which may be helpful in assessing at-risk populations. These studies can control the amounts and types of plastic ingested, including chemical-laden plastic, as well as track weathering, dosage, and components of the introduced items. Researchers can concurrently track changes in feeding, weight, growth rates, and other behaviors to gain a better understanding of how marine megafauna might be affected, which can ultimately be used to infer possible population-level impacts where interaction rates are well documented or believed to be high.

We recommend a strong emphasis on thorough veterinary examinations of live animals and necropsies of dead animals. The development of a global database of effects of plastic pollution from health assessments and necropsies would help provide information on the extent and frequency of plastic interactions with marine megafauna (Nelms et al. 2015).

*Modeling and assessment of long-term impacts from sub-lethal effects*

Although plastic pollution can lead to both lethal and sub-lethal effects, the latter are more difficult to identify and may be more prevalent and possibly even have broader population-level implications than lethal effects (Hoarau et al., 2014; Gall and Thompson 2015). Many of the threats that are commonly considered to be sub-lethal could become lethal if chronic and may have population consequences. Such sub-lethal effects could negatively affect an individual's survival probability, growth rate, reproductive output, and offspring survival. These changes to vital rates and their relative effects on population dynamics are in urgent need of future research.

Linking sub-lethal effects with measurable fitness consequences, such as reduced energy acquisition and assimilation, increased energetic demands, and potentially harmful behavioral changes, from laboratory or field-based research will allow researchers to develop models that can assess long-term impacts in individuals and ultimately populations. Currently, these consequences have only been documented

in a handful of studies that have focused on increased hydrodynamic drag, physiological stress, and nutrient dilution, with associated energetic demands, energy acquisition and assimilation, seemingly detrimental behavioral changes, and reproductive impairment (e.g. McCauley & Bjorndal 1999; Snoddy et al. 2009; Snoddy and Williard 2010; Van der Hoop et al. 2017). For example, physiological stress and other health indices can be quantified by evaluating blood chemistry, either in the laboratory or field. Fitness consequences and survivorship can then be estimated using tagging technology coupled with known physiological stress levels upon release of the animal. If recaptured, blood samples and a range of other health indices can be taken. This approach has been used to assess short and long-term impacts of catch and release fishing on sharks and other large pelagic fishes, where regression models have linked blood chemistry and angling time to infer short and long-term post-release survivorship based on the magnitude of pH change (Skomal 2007).

Although it currently will be challenging to achieve meaningful sample sizes, future field studies might combine tagging or telemetry techniques with physiological analyses to measure or infer post-plastic-interaction survival rates, growth rates, reproductive output, and health status for individual animals. For example, post-release mortality in juvenile sea turtles entangled in gillnets off the North Carolina coast was documented using both satellite telemetry and analysis of blood biochemistry (Snoddy & Williard 2010). With advances in tagging technology, it will become logistically easier to assess the extent to which sub-lethal effects may become lethal or result in reproductive impairment.

## Conclusions and recommendations

Despite increased interest in and awareness of the presence of plastic pollution throughout the world's oceans, our review underscores a dearth of available empirical information for informing demographic assessments of impacts on marine megafauna. Understanding plastic pollution in population-level contexts will allow for prioritization of limited conservation resources among threats affecting the same populations in the same areas. As marine habitats and prey items continue to become saturated in the face of increasing plastic pollution worldwide, population-

level effects in marine megafauna may increase and become easier to assess. Nevertheless, plastic pollution has clearly led to many animals suffering slow and painful deaths, which raises serious concerns for animal welfare (Votier et al. 2011). Potential solutions to hazardous plastic in the environment are as complex as for any other pollutant, involving sociopolitical aspects of human behavior change as well as engineering solutions to escape during transport, inefficiency of waste collection and disposal, and alternative materials (Gold et al. 2013). Although these solutions are outside the scope of our review, we point to avenues of investigation that would inform solutions benefitting marine megafauna specifically.

One important gap lies in understanding the origins of plastic pollution that pose a hazard to marine animals. Forensic investigation into errant plastic have revealed general source points and original usage categories (Woodall et al. 2015), but this work is on a miniscule scale relative to the global scope of plastic pollution. Conversely, data on plastic waste mismanagement by country (Jambeck et al. 2015) provides information on a broad scale, but does not identify hazard origins relative to marine habitats. Modeling of ocean surface currents has the potential to describe geographic origin of plastic pollution in drift patches (Van Sebille et al. 2012), which can identify human population centers for outreach and technology transfer. Plastic pollution sources might also come from identifying original usage. Original use identification could be as direct as matching shapes, colors, and lettering of plastic in marine habitats to cataloged items, and as inferential as assuming use applications based on resin identification from spectroscopy (Zettler et al. 2013; Rocha-Santos & Duarte 2015).

Comprehensive efforts to better understand and mitigate the effects of plastic pollution on marine species and ecosystems worldwide are urgently needed. Mitigation can be achieved in part by reducing the use of disposable and short-lived plastic items and more effective recycling programs (Hopewell et al. 2009). Reducing the exposure of marine megafauna to plastic will require lowering the plastic loading rate. Based on studies of the origin of plastic pollution cast on marine beaches (Pruter 1987; Derraik 2002) and at sea (Ryan et al. 2009), there are many sources. Identifying major origins of plastic pollution would guide public outreach efforts, enforcement, and export of trash management technology and methods. Re-

designed or modified fishing gear, coupled with policy initiatives that include economic incentives or deterrents, should be developed as a means to reduce gear loss and discarding at sea (Wilcox et al. 2016).

Finally, we highlight and encourage the multidisciplinary nature of potential solutions to threats from marine plastic pollution. Ocean research is not likely to result in information helpful for reducing this threat without work coordinated between resource experts, oceanographers, sociologists, materials scientists, and specialists in achieving human behavior change.

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**Dataset S1 [Excel file].** Dataset of all published, peer-reviewed studies on entanglement in (tab 1) and ingestion of (tab 2) plastic pollution by air-breathing marine megafauna between 1969 and 2018; percentage mortality is attributed to entanglement in or ingestion of plastic. Tab 3 includes the number and percentage of published, peer-reviewed plastic pollution studies conducted on marine megafauna species between 1969 and 2018.

## Appendix 3: Research dissemination

During my PhD, I have disseminated my research through peer-reviewed publications, oral and poster presentations at international and domestic conferences and public outreach events:

### Peer-reviewed publications

- **Nelms SE**, Coombes C, Foster LC, Galloway TS, Godley BJ, Lindeque PK, Witt MJ (2017) Marine anthropogenic litter on British beaches: A 10-year nationwide assessment using citizen science data. Science of the Total Environment. DOI:10.1016/j.scitotenv.2016.11.137
- **Nelms SE,** Galloway TS, Godley BJ, Jarvis DS, Lindeque PK (2018) Investigating microplastic trophic transfer in marine top predators. *Environmental Pollution*. DOI: 10.1016/j.envpol.2018.02.016
- **Nelms SE**, Barnett J, Brownlow A, Davison NJ, Deaville R, Galloway TS, Lindeque PK, Santillo D, Godley BJ (2019) Microplastics in marine mammals stranded around the British coast: ubiquitous but transitory? *Nature Scientific Reports.* DOI: 10.1038/s41598-018-37428-3
- **Nelms SE**, Parry HE, Bennett KA, Galloway TS, Godley BJ, Lindeque PK (In review) What goes in, must come out: combining scat-based molecular diet analysis and quantification of ingested microplastics in a marine top predator, the grey seal (*Halichoerus grypus*)
- Senko JF, **Nelms SE**, Reavis J, Witherington B, Godley BJ, Bryan P (In review) Global impacts of plastic pollution on air-breathing marine megafauna: A review with new research priorities

### Awards and grants

- Named on the winning NERC Societal Impact Award | NERC Impacts Awards 2018
- Employed as a Graduate Research Assistant through a research grant (£7,400) to investigate anthropogenic litter in Marine Protected Areas (MPAs) | University of Exeter and Natural England (2018 - 2019)

- Fully-funded attendance at the International Training Workshop on Microplastic Debris, Peru 2018 | Newton-Paulet Fund/ British Council (2018)
- Travel grant to attend the 22nd Biennial Conference on the Biology of Marine Mammals, Halifax, Canada (2017)
- Best poster award (Changing Planet theme) | NERC Wessex Congress (2017)
- Best poster award | Plymouth Marine Science & Education Foundation (PlyMSEF) conference (2017)
- Best poster award | NERC GW4+ Associate Partners event (2017)
- Runner up award for Best Poster | British Ecological Society Annual Meeting (2016)

**Conference presentations and seminars**

- Oral: 'Marine litter, microplastics and marine mammals' | Scottish Oceans Institute (University of St. Andrews) seminar series 2018
- Oral: 'Microplastics in marine mammals' | Marine Alliance for Science and Technology for Scotland (MASTS) Annual Science Meeting 2018
- Oral: 'Microplastics in marine mammals' | NERC Wessex Congress 2018
- Poster: 'Microplastics in marine mammals' | 22nd Biennial Conference on the Biology of Marine Mammals 2017
- Poster: 'Investigating microplastic trophic transfer in a marine top predator' | NERC Wessex Congress 2017
- Poster: 'Investigating microplastic trophic transfer in a marine top predator' | PML – University of Exeter Science Day 2017
- Poster: 'Investigating microplastic trophic transfer in a marine top predator' | PlyMSEF Conference 2017
- Poster: 'Investigating microplastic trophic transfer in a marine top predator' | NERC GW4+ Associate Partners event 2017
- Poster: 'Investigating microplastic trophic transfer in a marine top predator' | British Ecological Society Annual Meeting 2016
- Oral: 'Marine anthropogenic litter on British beaches: A 10-year nationwide assessment using citizen science data' | Plymouth Marine Laboratory seminar series 2016

- Oral: 'Marine anthropogenic litter on British beaches: A 10-year nationwide assessment using citizen science data' | Marine Ecology and Conservation Network forum 2016
- Oral: 'Marine anthropogenic litter on British beaches: A 10-year nationwide assessment using citizen science data' | MICRO2016, Lanzarote
- Oral: 'Marine anthropogenic litter on British beaches: A 10-year nationwide assessment using citizen science data' | PML – University of Exeter Science Day 2016
- Oral: 'Marine anthropogenic litter on British beaches: A 10-year nationwide assessment using citizen science data' | Lecture for MSc Applied Marine Science students, Plymouth University 2016

**Policy contributions**

- Cited in 'Future of the Sea: final report', UK Government Office for Science (2018)
- Cited in 'Microplastics in fisheries and aquaculture', Food and Agriculture Organization of the UN (2017)
- Cited in 'The environmental impacts of marine litter', UK Marine Management Organisation (2017)
- Contributed to UK Environmental Audit Committee 'Environmental impact of microplastics' inquiry (2016)

**Public outreach**

*Talks*

- 'Microplastics in marine mammals stranded around the British coast' | Marine Strandings Network Forum (Cornwall Wildlife Trust) 2019
- 'The global impacts of plastic pollution on marine wildlife' | Marine plastic pollution: the science story (Zoological Society of London) 2019
- Tale of the Turtle and the Plastic Jellyfish children's book reading | Greyfriars Roman Catholic Primary School, St. Andrews 2018
- Tale of the Turtle and the Plastic Jellyfish children's book reading | Mayflower Community Academy's Support Centre, Plymouth 2018

- ‘The problem with plastic’ | Falmouth Marine Conservation Group public event, Penryn 2018
- ‘The problem with plastic’ | Field Studies Council Research Seminar, Slapton Ley Field Centre 2017
- Tale of the Turtle and the Plastic Jellyfish children’s book reading (Skype) | Humberstone Junior Academy 2017
- ‘The problem with plastic’ | World Oceans Day event, Environment Agency 2017
- ‘The problem with plastic’ | Plymouth Rotary Club 2016

*Magazines articles and books*

- ‘Sea turtles and the perils of plastic’ article | Current Conservation
- ‘Seal poo unravels the microplastic journey through marine food webs’ article | The Science Breaker
- ‘Plastic pollution: Not just a drop in the ocean’ article | Biosphere Magazine
- ‘The Tale of the Turtle and the Plastic Jellyfish’ children’s book

*Documentaries*

- ‘Plastic Britain: On our watch’
- ‘A Plastic Wave’

*Other*

- Invited to attend a Plastic Free Coastlines event in Westminster | Surfers Against Sewage 2017
- Showcased research group’s work to Princess Anne during a royal visit to Plymouth Marine Laboratory 2017
- Assisted at a Women in STEMM careers event | Devonport High School for Girls, Plymouth 2016
- Attended a multi-stakeholder marine litter workshop | University of Exeter 2015