The Impact of Plastic Pollution on Marine Turtles

Submitted by

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

Signed:

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Ingested plastic from a juvenile green turtle (*Chelonia mydas*) from Northern Cyprus

Abstract

Plastic debris is entering into the marine environment at an accelerating rate, now becoming one of the most ubiquitous and long-lasting changes in natural systems. Marine turtles are large marine vertebrates with complex life histories and highly mobile behaviour that may make them particularly vulnerable to its impacts. The main goals of this thesis were to i) evaluate the potential implications of the presence of plastic pollution in the environment to marine turtles by reviewing current literature ii) provide a global summary of the issue of entanglement in this taxon, utilising a global network of experts iii) explore the drivers of key interactions between marine turtles and plastic ingestion and develop novel additions classification methodologies to explore selective ingestion of plastics iv) develop a methodology for investigating and isolating the presence of microplastic ingestion in marine turtle gut content and v) examine plastic pollution on a key habitat for marine turtles e.g. nesting beaches. Major findings of the thesis include i) the issue of entanglement with plastic debris, the majority in ghost fishing gear, is both an under-reported and under-researched threat ii) a clear display of strong diet-related ingestion towards plastic debris that resemble natural food items, utilising a case study of green turtles in Northern Cyprus iii) a method development that allowed the identification and isolation of a suite synthetic particles in gut content residue samples, providing evidence of ingestion of synthetic debris at the microscopic size class iv) a more comprehensive viewpoint on plastic concentrations on nesting beaches, in the form of 3D sampling to investigate subsurface plastic densities, showing microplastics present down to turtle nesting depth of both loggerhead and green turtles in Northern Cyprus. In conclusion, this thesis forms the most detailed and comprehensive investigation to date on the impacts of this pollutant on the taxon of marine turtles; contributing to

knowledge into macro and microplastic ingestion, entanglement and key habitats through method development and integration of marine turtle feeding ecology and developmental biology.

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Table of contents

List of tables and figures

Chapter 2: Plastic and marine turtles: a review and call for research

Chapter 3: A global review of marine turtle entanglement in anthropogenic debris: A baseline for further action

Table 1. Summary of all studies on entanglement of marine turtles in plastic debris. CCL: curved carapace length (cm); na: not available

Table 2. Summary of major challenges regarding marine turtle entanglement as listed by respondents

Table 3. Summary of research needs regarding marine turtle entanglement as listed by respondents

Table 4. Summary of priority actions regarding marine turtle entanglement as listed by respondents

Figure 1. Impacts of marine turtle entanglement: (a) live leatherback turtle entangled in fishing ropes which increases drag, Grenada 2014 (photo: Kate Charles, Ocean Spirits); (b) drowned green turtle entangled in ghost nets in Uruguay (photo: Karumbe); (c) live hawksbill turtle entangled in fishing material constricting shell growth, Kaeyama Island, Japan 2001 (photo: Sea Turtle Association of Japan); (d) live hawksbill turtle with anthropogenic debris wrapped around front left flipper constricting usage of limb which could lead to amputation and infection, Kaeyama Island, Japan 2015 (photo: Sea Turtle Association of Japan). All photos used with express permission

Figure 2. Inter-species comparison of the proportion of: (a) stranded individuals found entangled and (b) individuals found alive when discovered entangled. Violin plots show the kernel density of data at different values. Median (black dot) with interquartile range boxplot (black/white) and grand median (black dashed line). Turtle species abbreviations: CC: loggerhead *Caretta caretta*; CM: green *Chelonia mydas*; DC: leatherback *Dermochelys coriacea*; EI: hawksbill *Eretmochelys imbricata*; LK: Kemp's ridley *Lepidochelys kempii*; LO: olive ridley *Lepidochelys olivacea*; ND: flatback turtle *Natator depressus*

Figure 3. Inter-specific comparison of the breakdown of entangled sea turtle species by life stage. Black: pelagic juveniles (PJ); white: neritic juveniles (NJ); light grey: juveniles (JV); dark grey: adults (AD); see Fig. 2 for species abbreviations. Flatback turtles were only categorised into juvenile or adult classes with advice from species experts. Sea turtle skull figures used with permission of WIDECAST; original artwork by Tom McFarland

Figure 4. Entangling materials. L/DF: lost/discarded fishing; A/F: active fishing; Non-F: non fishing; Poly sheet: poly - ethylene sheeting. Black: common (10% or more of cases); grey: sometimes (less than 10% of cases); white: never. Not all participants categorised each material; total number of responses for each material shown on the right of the graph

Figure 5. Responses to comparison of other threats faced by marine turtles compared to entanglement (n = 63). Black: greater than entanglement; grey: similar threat; white: less than entanglement; striped: unsure

Chapter 3: Supplementary Information

Box S1. First Questionnaire

Box S2. Second Questionnaire

Figure S1. Likelihood of population level effects. Number of responses from experts when asked how likely entanglement in man-made debris to causing population level effects in marine turtles (n=63).

Chapter 4: Diet-related selectivity of macroplastic ingestion in marine turtles

Figure 1. Macroplastic ingestion in green turtles (*Chelonia mydas*) from the Eastern Mediterranean. a) Ingested plastic removed from the intestine of a juvenile (CCL=33cm) showing the high quantities and diversity of plastic debris ingested. b) Curved carapace length (cm) vs. the number of ingested pieces of plastic (n=19).

Figure 2. Marine turtle diet-related selectivity in macroplastic ingestion in the green turtles (*Chelonia mydas*) (n=34). Manly's Selectivity Ratios. A value >1 this indicates a positive selectivity for that type/colour category than availability in the environment. Error bars indicate 95% confidence intervals. a) type of plastic debris SHE=sheetlike plastics, THR=threadlike plastics, FOAM= foamed plastics, FRAG= hard plastics, POTH= other 'plastic like' items, IND= industrial nurdles b) colour of plastic debris. Cl=Clear, Blk=Black, Y=Yellow, Wh=White, Gn=Green, Bl=Blue, Br=Brown, Gy=Grey, O=Orange, P/P=Pink/Purple, R=Red. c) width/length ratio. If the ratio

number produced was <0.2 this represented a rectangular shape whereas a ratio close to 1 indicated a more square or circular piece of debris.

Chapter 4: Supplementary Information

Figure S1. Type, colour and size of plastic debris from beach surveys (n=1167) a) type of plastic debris SHE=sheetlike plastics, THR=threadlike plastics, FOAM= foamed plastics, FRAG= hard plastics, POTH= other ‘plastic like’ items, IND= industrial nurdles b) colour of plastic debris. Cl=Clear, Blk=Black, Y=Yellow, Wh=White, Gn=Green, Bl=Blue, Br=Brown, Gy=Grey, O=Orange, P/P=Pink/Purple, R=Red. c) width/length ratio. If the ratio number produced was <0.2 this represented a rectangular shape whereas a ratio close to 1 indicated a more square or circular piece of debris.

Chapter 5: Microplastic ingestion ubiquitous in marine turtles

Figure 1. Study sites and number of each species sampled; Embedded pie charts of proportion of individuals with macroplastic ingestion (%); white=absent, black=present. Left to right: Atlantic (North Carolina, USA), Mediterranean (Northern Cyprus), Pacific (Queensland, Australia). Species codes: CC= loggerhead turtle (*Caretta caretta*), CM= green turtle (*Chelonia mydas*), DC= leatherback turtle (*Dermochelys coriacea*), LK= Kemp’s ridley turtle (*Lepidochelys kempii*). ND= flatback turtle (*Natator depressus)*, EI= hawksbill turtle (*Ertmochelys imbricata*) and LO= olive ridley turtle (*Lepidochelys olivacea).* Sea turtle skull figures used with permission of WIDECAST; original artwork by Tom McFarland

Figure 2. Synthetic micro-particle ingestion in all species of marine turtles from three ocean basins. Total number of particles identified in each sample per species per ocean basin. Black line =mean number of particles. Note that 100ml was analysed per animal irrespective of size, so the number of particles per animal should not be over-interpreted. ATL= Atlantic (North Carolina, USA) loggerhead turtle (*Caretta caretta*, n=8), green turtle (*Chelonia mydas,* n=10), leatherback turtle (*Dermochelys coriacea,* n=2), kemp's ridley turtle (*Lepidochelys kempii* n=10). MED= Mediterranean (Northern Cyprus) loggerhead turtle (n=22), green turtle (n=34). PAC= Pacific (Queensland, Australia) loggerhead turtle (n=3), green turtle (n=7), flatback turtle (*Natator depressus,* n=4), hawksbill turtle (*Eretmochelys imbricata,* n=1) and olive ridley turtle (*Lepidochelys olivacea,* n=1). Sea turtle skull figures used with permission of WIDECAST; original artwork by Tom McFarland

Figure 3. Type and colour of synthetic particles including microplastics identified from marine turtle gut content. Mean (±S.E.) percentage make up of each type (fibre, fragments, beads) isolated within the gut content residue samples from stranded turtles from the Atlantic (white), Mediterranean (light grey) and Pacific (dark grey). Colours categorised for fibrous synthetic particles ATL=Atlantic, MED= Mediterranean and PAC=Pacific. X= no-detections

Chapter 5: Supplementary Information

Table S1. Summary of marine turtles (n=102) by sites, species, size (CCL: Curved Carapace Length cm; notch to notch), % macroplastic and synthetic particle ingestion presence. U=unmeasured due to damage

Table S2. Results from the subsample of isolated particles (n=169) analysed using Fourier transform infrared spectroscopy (FT-IR) to determine their polymer make up from gut content residue samples of marine turtles

Figure S1. Enzymatic digestion of marine turtle gut content a) Stranded juvenile green turtle (CCL=33cm) from the North Cyprus coastline b) the gut content residue sample from the juvenile green turtle that has been enzymatically digested which has removed the majority of the biological material allowing the identification of suspected microplastics c) a microplastic fibre isolated from the gut content of the juvenile green turtle.

Chapter 6: The True Depth of the Mediterranean Plastic Problem: Extreme Microplastic Pollution on Marine Turtle Nesting Beaches in Cyprus

Figure 1. Mean microplastic in particles m^{-3} within turtle nesting line (TNL) surface samples (0-2cm), across numbered sample beaches with fitted standard error bars.Stack shades represent the three different coastlines in the map insert: Hatcheded= West (n=3, beach number 1-3), Grey = North (n=8, beach number 4-

11), White = East (n=6, beach number 12-17). Individual beach co-ordinates can be found in Table 1, supplementary data.

Figure 2. Grand mean (±S.E) of microplastic abundance in particles m^{-3} at different sand depths at turtle nesting areas (n=17 sites).

Figure 3. Microplastic weight/volume (g m^{-3}) classification categories on each beach (grey dots) (n=17). Black line = mean microplastic weight/volume (g m^{-3}) across all sample beaches cores.

Figure 4. Particle trajectories (mapped by receiving beach; n=17) rasterised to a 20 x 20 km grid resolution. Tracks per grid square are counted. To enable 'at sea' trajectories to be clearly displayed data within 5 km of the coast have been removed.

Figure 5. Drifter source locations (n=5487,mean ± s.e.) by country for monitored beaches (n=17). Countries are identified using their 2 digit sovereign state ISO code as follows: Greece (GR), Turkey (TR), Cyprus (CY), Syria (SY), Lebanon (LB), Israel (IL), Gaza Strip (GZ), Egypt (EG) and Libya (LY).

Chapter 6: Supplementary Information

Table S1. Microplastic levels across study beaches (n=17). Co-ordinates presented in DMS (Degrees, Minutes, Seconds). Mean values in particles m^{-3} and g.m^{-3} for the strandline (SL) and turtle nesting line (TNL).

Table S2. Drifter source locations (n=5487,mean ± s.e.) by country for monitored beaches (n=17).

Figure S1. Experimental design of beach sediment sampling: 10 paired samples taken along the turtle nesting line (○) and strandline (**x**), plotted using GPS locations of samples taken on beach 5. Samples positioned ~34m from beach ends and ~68m apart on the 680m long beach

Figure S2. Mean microplastic in g m^{-3} within turtle nesting line (TNL) surface samples (0-2cm), across numbered sample beaches with fitted standard error bars. Striped= West (n=3, beach number 1-3), Grey = North (n=8, beach number 4-11), White = East (n=6, beach number 12-17).

Figure S3. Grand mean (±S.E) of microplastic abundance in particles g^{-3} at different sand depths at turtle nesting areas (n=17 sites, n=170 sampling locations).

Figure S4. Maps the particle drifter source locations by monitored beach. These are locations where advected particles have become 'stuck' at their coastal 'sources'. Only locations that were within 5 km of the coast have been mapped

Author’s declaration

All chapters presented in this thesis were written by Emily M. Duncan, under the supervision of Annette C. Broderick, Tamara S. Galloway, Penelope K. Lindeque and Brendan J. Godley.

Macroplastic analyses was conducted at the Marine Turtle Research Group, Centre for Ecology and Conservation (CEC) of the College of Life and Environmental Sciences, Penryn Campus, University of Exeter, and gut content residue digestion for microplastic identification was carried out by Plymouth Marine Laboratory. Plastic polymer analyses was conducted at Greenpeace Research Laboratories, Innovation Centre Phase 2, University of Exeter. Access to Spotlight 400 imaging FT-IR microscope was made possible under a Research Partnership Agreement between the Greenpeace Research Laboratories and PerkinElmer. Fieldwork was carried North Cyprus, North Carolina, USA and Queensland, Australia under the coordination of Marine Turtle Conservation Project (MTCP) and Society of Protection of Turtles (SPOT), North Carolina Wildlife Resources Commission, College of Marine and Environmental Science, James Cook University, Townsville and the Department of Environment and Heritage Protection, Queensland Government; with numerous international volunteers contributing to the reporting and collection of stranding and bycaught marine turtles in each location.

Chapter 2: Plastic and marine turtles: a review and call for research

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EMD & SEN joint lead the literature review and data collection. EMD & SEN assembled and analysed data, produced all figures and was lead author on the manuscript. BJG, ACB, TSG and PKL provided guidance on data analysis and writing, and all co-authors provided useful comment on the manuscript.

Chapter 3: A global review of marine turtle entanglement in anthropogenic debris: A baseline for further action

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Chapter 4: Diet-related selectivity of macroplastic ingestion in marine turtles

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Chapter 5: Microplastic ingestion ubiquitous in marine turtles

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EMD all conducted fieldwork and sampling with assistance by WJF, MHG, MH, CJL, LCOM, RTES. EMD performed all sample processing and analysis under the guidance of TSG, PKL and DS. EMD assembled and analysed data, produced all figures and was lead author on the manuscript. BJG, ACB, TSG and PKL provided guidance on data analysis and writing, and all co-authors provided useful comment on the manuscript.

Chapter 6: The True Depth of the Mediterranean Plastic Problem: Extreme Microplastic Pollution on Marine Turtle Nesting Beaches in Cyprus

Emily M. Duncan, Jessica A. Arrowsmith, Charlotte E. Bain, Annette C. Broderick, Johnathon H. Lee, Kristian Metcalfe, Stephen K. Pikesley1, Robin T. E. Snape, Erik van Sebille, Brendan J. Godley.

EMD, JAA, CEB, JHL and RTES conducted fieldwork and sampling. EMD assembled and analysed field data, produced figures and was lead author on the manuscript. SKP and EVS produced particle drifter analysis and produced related figures. BJG, ACB, TSG, KM and PKL provided guidance on data analysis and writing, and all co-authors provided useful comment on the manuscript.

List of notations and abbreviations

Notations:

µm – micrometre

mm- millimetre

Abbreviations:

Macroplastic- plastic debris with length greater than 5mm

Microplastic- plastic debris with length less than 5mm

POPs/ PCBs – Persistent Organic Pollutants/ Polychlorinated biphenyls

Chapter 1. General Introduction

Plastic pollution and marine wildlife

Plastic debris is entering into the marine environment at an accelerating rate, now becoming one of the most ubiquitous and long-lasting changes in natural systems (Barnes et al. 2009, Jambeck et al. 2015). Extremely high densities are occurring along coastlines, in mid-oceanic gyres, on the seafloor, in the water column and in the surface of the oceans (Watts et al. 2015). Since plastic waste is not biodegradable, its high durability means it may persist for centuries (Barnes et al. 2009). Marine plastic pollution has now been estimated to interact with over 700 species (Gall & Thompson 2015). For many of these species it presents a major threat through ingestion, entanglement, the degradation of key habitats and wider ecosystem effects. Among these, species of large marine vertebrates, such as marine turtles, are particularly vulnerable to the impact of plastic pollution due to their complex life histories and highly mobile behaviour (Schuyler et al. 2014).

The ingestion of plastic debris is now a global phenomenon for numerous marine species. It is thought to be occurring in at least 43% of cetacean species, 36% of seabird species globally, many species of fish, and has been reported in all species of marine turtle (Campani *et al.* 2013). However to date the majority of studies have focused on macroplastics (>5mm), the ingestion of which by marine turtles has the potential to cause lethal effects including blockages, internal injuries and lacerations. In addition, they may cause adverse sub-lethal effects; dietary dilution that can lead to starvation, malnutrition and impaired immunity (Schuyler et al. 2014).

Alongside studies on this “macroplastic” pollution (>5mm), there has recently been a growing concern about “microplastics”; these are defined as plastic particles <5mm

(Andrady 2011). Primary microplastics are manufactured to be of microscopic size, these are most commonly recognised as “micro-beads” in cosmetics products but can also appear as fibres from clothing and abrasives for jet washing (Derraik 2002). Secondly, microplastics are fragments derived due to the breakdown of large “macroplastics” within the marine system, this being the result of wave action, exposure to UV radiation and physical abrasion (Browne et al. 2007). Due to their abundance and bioavailability these micro plastics have been considered as a pollutant in their own right (Cole et al. 2011).

Finally entanglement in plastic debris, such as that derived from land-based sources and lost or discarded fishing gear (“ghost nests”) is now recognized as a major threat for many marine species (Vegter et al., 2014). To such a degree that the Marine Strategy Framework Directive (MSFD) Technical Subgroup on Marine litter has announced that they are developing a dedicated monitoring protocol for their next report (MSFD GES Technical Subgroup on Marine Litter, 2011). Plastic debris entanglement has the potential to cause a multitude of impacts such as serious wounds leading to maiming, amputation or death, increased drag, restricted movement or choking; overall leading to reduced fitness through starvation, infection or drowning (Lawson et al. 2015). However, quantitative summaries of this issue remain extremely limited for many marine species.

Overall, although plastic pollution is capable of having numerous deleterious impacts on vulnerable marine species such as marine turtles our understanding of the underlying mechanisms driving these phenomena, the presence and level of novel pollutants on the plastics and quantitative data on the scale of these issues remain extremely limited within marine turtles.

Extant marine turtle species and current conservation status

Globally there are seven species of extant sea turtles. The majority (six) are found in the family of Cheloniidae; the green (*Chelonia mydas*), loggerhead (*Caretta caretta*), kemp's ridley (*Lepidochelys kempii*), hawksbill (*Eretmochelys imbricata*), flatback (*Natator depressus*) and the olive ridley (*Lepidochelys olivacea*) turtle. The other family of Dermochelyidae only contains a single species the leatherback turtle (*Dermochelys coriacea*).

Many populations of the marine turtle species have experienced significant declines within the past century due to numerous threats for examples habitat destruction, direct exploitations and incidental capture from fisheries and climate change (Hamann et al. 2010, Rees et al. 2016). This suite of synergistic threats are ever evolving most recently to include the impacts of marine plastic pollution (the basis of this thesis) (Nelms et al. 2016). All of these have the capacity to vastly alter population numbers and geographic distributions. For this reason they are internationally recognised of species of conservation concern and are currently included in the 2018 IUCN Red List (Status and population trend of each species summarised in Table 1.).

However there is an importance to consider the assessment of separate populations when regarding extreme vulnerability in some of these worldwide, which requires more flexible assessment frameworks to reflect this globally. Therefore there has been a recent movement towards the construction of regional management units to take in account the large of variation among species and regions (Wallace et al.

2011). Overall initial assessments show average values of population risk and threats criteria showed globally long-term population trends were declining on average across marine turtles subpopulations however in more recent years they have begun to stabilise or increase (Wallace et al. 2011). In terms of spatial differences between ocean basins, sub-populations in the Pacific Ocean have the highest average population viability risk values in contrast to the sub-populations in the Atlantic Ocean and Mediterranean Sea that experiences the highest threats scores. Whereas the Indian Ocean sub-populations has the highest data uncertainty in both population viability and threats scores. In terms of species over 40-50% of hawksbill, loggerhead and leatherback subpopulations are at both classified “high risk-high threats” with lower percentages of the other species sub-populations (Wallace et al. 2011).

Species	Status	Population trend
Green (*Chelonia mydas*)	Endangered	Decreasing
Loggerhead (*Caretta caretta*)	Vulnerable	Decreasing
Olive Ridley (*Lepidochelys olivacea*)	Vulnerable	Decreasing
Kemp’s Ridley (*Lepidochelys kempii*)	Critically endangered	NU
Hawksbill (*Eretmochelys imbricata*)	Critically endangered	NU
Leatherback (*Dermochelys coriacea*)	Vulnerable	Decreasing
Flatback (*Natator depressus*)	Data deficient	NU

Table 1. The IUCN Red List status and population trend marine turtle species. NU=needs updated

Marine turtle life cycle

Marine turtles have complex life history patterns encompassing terrestrial habitats, neritic (coastal; water depths that do not exceed 200m) zone and oceanic (open ocean) zone (Bolten 2003). Inter-specific differences with life history patterns exist between the seven extant species with variable characteristics in the developmental and adult foraging stage (Omeyer et al. 2017). Hatchlings of all species emerge from the nest and enter into a “swim frenzy”. By definition a hatchling becomes a post-hatchling when it begins to feed, entering into the oceanic zone (Boyle 2006).

Hereby the contrast occurs, the majority of species; green (*Chelonia mydas*), loggerhead (*Caretta caretta*), kemp’s ridley (*Lepidochelys kempii*), hawksbill (*Eretmochelys imbricata*) and some populations of olive ridley (*Lepidochelys olivacea*) complete an early development in the oceanic zone and juvenile development in the neritic zone exhibiting an intermediate life history pattern (Bolten 2003). Post-hatchlings inhabit the oceanic waters for undetermined period time, feeding on epipelagic prey, where upon reaching a size threshold (Bjorndal et al. 2000, Bolten 2003),large juveniles recruit to neritic waters, undergoing a shift in their dietary composition (Omeyer et al. 2017). The exceptions to this type of life history are the flatback (*Natator depressus*) which has a completely neritic development, never entering into the oceanic zone and the leatherback (*Dermochelys coriacea*) and some olive ridley populations which have a life history pattern completely characterised by developmental and adult stages occurring in the oceanic zone (Bolten 2003, Boyle 2006, Omeyer et al. 2017, Wildermann et al. 2017).

Currently sexual maturity of sea turtles is thought to occur a certain age-sixe trade off, however this remains to be fully understood (Omeyer et al. 2017). Once this has occurred mature adult turtles will begin a pattern of migration from foraging to mating/nesting grounds; depending on species and population remigration intervals are believed to be to be between 2-4 years. These migrations are some the largest observed in marine megafauna worldwide (Jeffers & Godley 2016). Female marine turtles will then return to their natal beach (from once they hatched) to lay her eggs; the numbers of clutches and eggs within nests varying widely depending on the species and population. The female will remain in an interesting habitat until finally making the migration back to the foraging grounds. After a period of incubation the hatchlings will emerge from the eggs and hereby the whole cycle beings again. This is summarised in detail within Figure 1. (Omeyer et al. 2017).

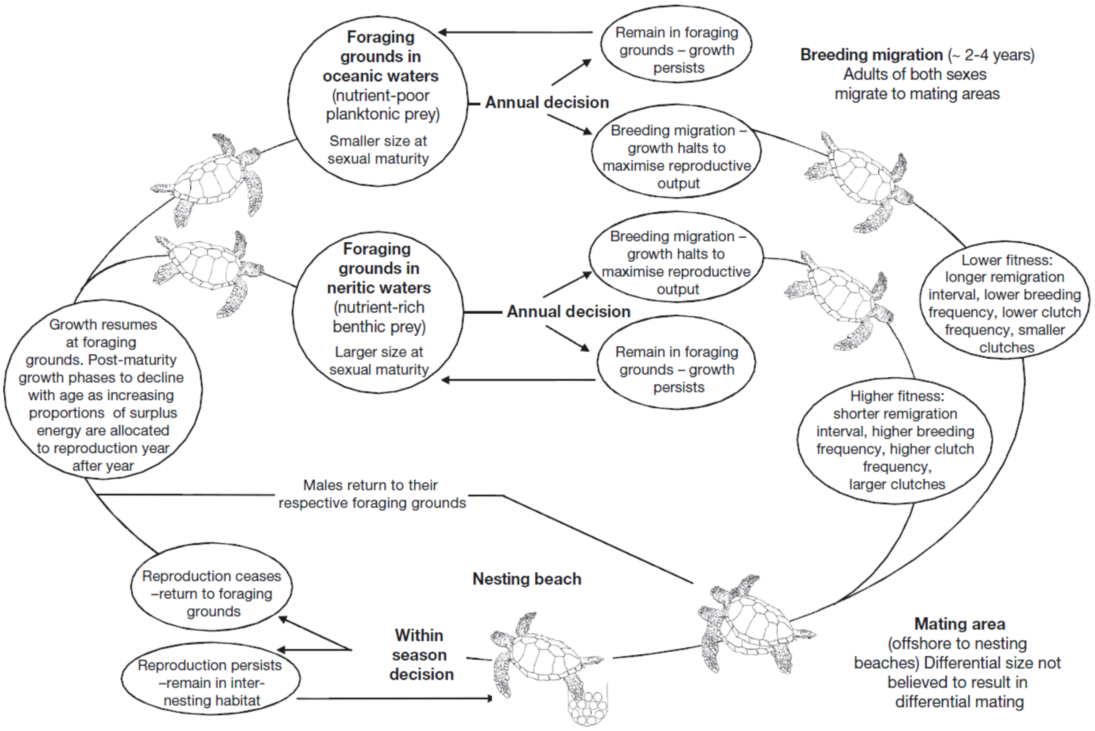

Fig 1. Dichotomous adult life cycle for loggerhead, green, hawksbill, Kemp’s Ridley and olive ridley turtles (Omeyer et al. 2017) Permission from author for use.

In the present thesis, **‘Investigating the impacts of plastic pollution on marine turtles’**, I explore the impacts of plastic pollution on marine turtle species and assess the magnitude to which it may present a threat through macro and microplastic ingestion, entanglement and degradation of key habitats such as nesting beaches.

In **Chapter 2**, **‘Plastic and marine turtles: a review and call for research’**, I review the evidence for the effects of plastic debris on turtles and their habitats, highlight knowledge gaps, and make recommendations for future research. Marine turtles are particularly vulnerable due to their use of a variety of habitats, migratory behaviour, and complex life histories leaving them subject to a host of anthropogenic stressors. By compiling and presenting this evidence, I 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.

In **Chapter 3**, **‘A global review of marine turtle entanglement in anthropogenic debris: A baseline for further action’**, I provide a global summary of the issue of entanglement in this taxon; including a literature review and expert opinions from conservation scientists and practitioners worldwide. I report on entanglement encounter rates in terms of species, ocean basins and life stages, in addition to exploring the materials that contribute to the majority of reported entanglements. Surveyed experts were also asked to consider whether this threat was having a population level effect in some areas of the world, as well as to comment on the challenges, research needs and priority actions facing marine turtle entanglement.

In **Chapter 4**, **‘Diet-related selectivity of macroplastic ingestion in marine turtles’**, I explore the drivers of key interactions between marine turtles and plastic by developing novel additions to classification methodologies that allow us to test the hypothesis that plastic is selectively ingested when it resembles food items of green turtles (*Chelonia mydas)*. Using ingested macroplastic type, colours and shapes when compared to the environmental baseline of plastic beach debris I test for selective ingestion of macroplastics. Furthermore the relationship between size of turtle (curved carapace lengths cm) and number/ mass of plastic pieces ingested is discussed with possible explanations from known feeding ecology and developmental biology. I call for further species specific visual recordings that would give greater insight into the selectivity of sea turtles in relation to ingested plastics based on a variety of physical properties.

In **Chapter 5**, **'Microplastics ubiquitous in multiple species of marine turtles in three ocean basins'**, I develop a method to investigate microplastic ingestion in marine turtles at three study sites (USA, Cyprus, Australia) by utilising an optimised enzymatic digestion technique previously used on zooplankton. This technique removes biological material from sea turtle gut content aiding the isolation of potential microplastics. I discuss the type, colour and size of synthetic particles as well as the polymer/material make-up of isolated particles. Finally, I suggest potential ingestion pathways in relation to marine turtle ecology and habitat use and provide recommendations for the use of this methodology for other large marine vertebrate species.

In **Chapter 6**, **'The True Depth of the Plastic Problem: Extreme Microplastic Pollution on Mediterranean Marine Turtle Nesting Beaches'**, I examine microplastic pollution on a key habitat for marine turtles, the nesting beach. I use North Cyprus as a case study; investigating the spatial variation in distribution of microplastics between beaches and coasts, classifying microplastics recovered as well developing a novel to quantify microplastics in sediment at sea turtle nest depth.

I rank our results compared to other global abundances of microplastic in beach sediments; discussing the potential repercussions for marine turtle populations. Finally I use particle drifter analysis hindcast modelling to suggest the likely major sources of plastic origin.

Overall this research is very timely given the current interest in this topic in the research community, general public and media. The overarching aim was to quantify and assess the potential threats and impacts the presence of the marine plastics in the environment may be having on marine turtles.

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Chapter 2. 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 fear is growing over the scale of threat, 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. 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, lesions, 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.

Introduction

Between 1950 and 2015, the total annual global production of plastics grew from 1.5 million tonnes to 299 million tonnes (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 principle 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, Reisser et al. 2014b, Wabnitz and Nichols, 2010). 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 (Cózar 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 (Cózar 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 less than 5mm in diameter), can lead to the accumulation of contaminants, such as heavy metals and polychlorinated biphenals (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 at an individual and potentially population scale through ingestion, entanglement, the degradation of key habitats and wider ecosystem effects (Barnes et al. 2009, Gall and Thompson, 2015; Vegter et al. 2014). Among these species are the marine turtles, whose complex life-histories and highly mobile behaviour can make them particularly vulnerable to individual exposure and therefore the creation of population impacts due to 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 risk from an individual mortality to population level declines, faced by key species (Vegter et al. 2014). Understanding these impacts 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).

This study carries 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, ISI Web of

Knowledge and Google Scholar was searched for the terms *plastic, plastic pollution, marine debris, marine litter, ingestion, entanglement, entrapment, ghost nets, 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 the word *turtle* was also included. 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). The review is structured in five major sections looking at 1) ingestion 2) entanglement 3) impacts to nesting beaches and 4) wider ecosystem effects and then suggest priorities for 5) 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 macro-algae they target directly (DiBeneditto and Awabdi, 2014). Alternatively, plastic ingestion may be a case of mistaken identity. As turtles are primarily visual feeders, they may mis-identify items, such as shopping bags, plastic balloons and sheet plastic, as prey and actively select them for consumption (Gregory, 2009, Hoarau et al. 2014; Mrosovsky, 1981; Tomás et al. 2002). 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, Schuyler et al. 2012; Swimmer et al. 2005). A number of studies have found that white and transparent plastics are the most readily consumed colours (Camedda et al. 2014, Hoarau et al. 2014, Schuyler et al. 2012, Tourinho et al. 2010). It is not certain, however, whether this pattern is a result of selectivity by the turtles or due to the differing proportions of plastic types and colours in the environment (Camedda et al. 2014, Schuyler et al. 2012). Aside from visual cues, it is possible that 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 microplastic 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 (Eriksson and Burton, 2003, McMahon et al. 1999). 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 individualsub-lethal effects that are difficult to identify, quantify and attribute to plastic ingestion as opposed to other water quality issues (Baulch and Perry, 2014, Gall and Thompson, 2015, Vegter et al. 2014). These are discussed later in this section.

As with many other taxa, 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 intra-species 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 downwelling areas (Bolten, 2003, Boyle et al*.* 2009, Scott et al. 2014). These areas can be highly productive and act as foraging hotspots for many marine taxa including fish, sea birds and marine turtles (Scales et al*.* 2014, Schuyler et al*.* 2014, Witherington, 2002). However, along with food, advection also draws in and concentrates floating anthropogenic debris, increasing the likelihood of exposure to plastic. This spatial overlap potentially creates an ecological trap for young turtles (Battin, 2004, Carr, 1987, Cózar et al*.* 2014, Tomás et al*.* 2002, Witherington et al*.* 2012). Their exposure 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 (Hoarau et al*.* 2014, McCauley and Bjorndal, 1999, Schuyler et al*.* 2012). 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, 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 (Arthur et al*.* 2008, Hawkes et al*.* 2006, Schuyler et al*.* 2012, Tomás et al. 2001, Witherington, 2002). 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 (González Carman et al*.* 2014). Indeed, González Carman et al. (2014) reported that 90% of the juvenile green turtles examined had ingested anthropogenic debris and postulated that, aside from the high concentrations of debris, poor visibility (caused by estuarine sediment) and therefore 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. 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%; Figure 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 observed pattern shows the need for caution when interpreting apparent patterns based on the number of observations of plastic ingestion among species.

The majority of research was carried out in the Atlantic Ocean basin (n=28 of 55 publications on plastic ingestion by turtles; Figure 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 inter-specific differences in feeding strategies, diet and habitat use that could result in varying likelihoods of exposure and consequences of plastic ingestion for individuals of each species (Bjorndal, 1997, Schuyler et al*.* 2014). For example, the generalist feeding strategy of loggerhead turtles seems to put it 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 sub-adults) (Bugoni et al*.* 2001, Hoarau et al. 2014, Tomás et al. 2001, 2002). This, though, may not mitigate the sub-lethal effects which may occur as a result of plastic ingestion (see Ecological effects section below). Although not heavily studied when compared to the other turtle species (Figure 1c), ingestion rates by Kemp's ridley turtles appear to be low. This may be because they specialise in hunting active prey, such as crabs, which plastic debris are 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 (Casale et al*.* 2008, Parker et al*.* 2005) 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 (DiBeneditto and Awabdi, 2014); another found 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 (León 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 reports that in 2003, a flatback 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 leatherbacks are also susceptible to plastic ingestion and Mrosovsky et al*.* (2009) estimated that approximately one third of all adult leatherbacks autopsied from 1968-2007 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 (Gall and Thompson, 2015; Hoarau et al*.* 2014, Schuyler et al. 2014). 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 (Derraik, 2002, Plotkin and Amos, 1990). 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 (Di Bello et al*.* 2013, Parga, 2012).

In some cases the sheer volume of marine 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, Santos et al. 2015, Schuyler et al. 2014). For example, Santos et al. (2015) found that only 0.5g 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 (Awabdi et al*.* 2013, Davenport et al. 1993). 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 of feeding stimulus and stomach capacity could lead to malnutrition through dietary dilution which occurs when debris items displace food in the gut, reducing the turtles 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, Tomás et al. 2002).

The presence of large quantities of buoyant material and the potential addition of trapped gas in the gut 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. However this remains to be tested. Additionally, plastic ingestion can also 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 Bisphenal-A and phthalates, leach out of ingested plastics and can be absorbed into the tissues of the animals, 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. 2011, Wright et al. 2013). Such contaminants have been shown to cause developmental and reproductive abnormalities in a number of taxa, such as egg-shell thinning and delayed ovulation in birds as well as hepatic stress in fish (Azzarello and Van Vleet, 1987, Oehlmann et al. 2009, Rochman et al. 2013, Vegter et al. 2014, Wiemeyer et al. 1993). To date, the knowledge base regarding these issues in marine turtles is limited.

Indirectly ingested micro-/nano plastics (although untested) may have the capacity to 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, Reisser et al. 2014a, Wright et al. 2013) and worthy of investigation.

It is possible that the sub-lethal individual 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 (Aguirre and Lutz, 2004, Landsberg et al. 1999). Stranded juvenile green turtles in Brazil exhibit both high occurrence of ingestion and incidences of this disease (Santos et al. 2011). However further studies are needed to clarify this. Additionally, plastic ingestion may impact health and weaken the turtle’s physical condition which could impair their 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 (Hazel and Gyuris, 2006, Hoarau et al. 2014, Lewison et al. 2004). 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 nets’), is now recognised as a major mortality threat to many marine species (Figure. 2c, Gregory, 2009, Vegter et al. 2014, Wilcox et al. 2013). Their sources are difficult to trace but their widespread distribution indicates that ocean currents and winds may be dispersal factors (Jensen et al. 2013, Santos et al. 2012, Wilcox et al. 2013). Entanglement is one of the major causes of turtle mortality in many areas including northern Australia and the Mediterranean (Camedda et al. 2014, Casale et al. 2010, Jensen et al. 2013, Wilcox et al. 2013). 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 referring to marine debris entanglement and turtles (Barreiros and Raykov, 2014, Bentivegna, 1995, Casale et al. 2010; Chatto, 1995; Jensen et al*.* 2013; Lopez-Jurado et al. 2003, Santos et al. 2012, Wilcox et al*.* 2013, 2014) and of these, seven are related to ghost fishing gear. For individual turtles, the effects of entanglement are injuries, such as abrasions, lesions, constriction or loss of limbs; a reduced ability to avoid predators or forage efficiently due to drag leading to starvation or drowning (Barreiros and Raykov, 2014, Gregory, 2009; 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 (Barreiros and Raykov, 2014, Chatto, 1995).

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, Stelfox et al. 2014, Wilcox et al, 2013, 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 (Gregory, 2009, Jensen et al. 2013, Matsuoka et al. 2005, Stelfox et al. 2014). Although this widespread problem is not unique to turtles, as a taxon, they appear to be particularly commonly impacted . 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 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 flatback turtle stranded in Australia after becoming trapped in 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 likely never published (B.

Godley, *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 sub-adults and adults (Santos et al. 2012). There could be several reasons for this. Firstly, the smaller size of young juveniles enhances their ability to escape. Secondly, 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, Witt et al*.* 2010). 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, Ivar do Sul et al. 2011, Özdilek et al. 2006, Triessnig, 2012). From large fishing nets to tiny microscopic particles, this debris presents a potential impact 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 (Chacón-Chaverri and Eckert, 2007). Alongside this, entanglement is a risk when debris, such as netting, mono-filament 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 (Özdilek 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 a higher number of male hatchlings than females within the clutch (Carson et al*.* 2011, Vegter et al*.* 2014, Witt et al*.* 2010). 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). However it is currently unknown at what abundance of plastic presence would be sufficient to effect these nest environment properties, further experimental studies are required. Other possible impacts include sediment contamination from absorbed persistent organic pollutants or leached plasticizers (Carson et al*.* 2011, Oehlmann et al*.* 2009, 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). However it remains untested

Wider ecosystem impacts

Marine turtles utilise 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 is outlined 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’ (Blumenthal et al*.* 2009, Goatley et al. 2012, León and Bjorndal, 2002, Sazima et al*.* 2010). 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 (Brown and Macfadyen, 2007, Matsuoka et al. 2005, 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 potentially 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 epi-biotic 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 sea floor (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 turtles. In terms of “risk” this can be defined here as exposure to plastic pollution and therefore “harm” to individuals via direct mortality or indirect sub-lethal effects. These are discussed below and summarised in Table 3.

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 the advocation 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 numerous 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 understanding 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 chance of exposure and therefore ingestion plastic debris. As such, these areas could act as a population sink (González Carman et al*.* 2014, Witherington, 2002; Witherington et al. 2012). As the development and survivorship of young turtles is critical for species persistence, it is important 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).

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 utilised (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 (Hoarau et al. 2014, Witherington, 2002). Integrating body condition indexes into necropsy practices, will generate a better understanding of the sub-lethal impacts of plastic ingestion, such as malnutrition and the absorption of toxins (Bjorndal et al*.* 1994, Gregory, 2009; Labrada-Martagón et al. 2010). It may also be useful to record conditions such as the presence of fibropapillomatosis or epi-biotic 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 is important to recognize that published studies do not necessarily represent a randomised 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 - 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. Rhers are strongly encouraged to publish both positive and negative results related to plastics and marine turtles.

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, seaturtle.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 conducted 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 standardised. 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 (Camedda et al*.* 2014, Schuyler et al. 2012). 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 (Hoarau et al*.* 2014, Lazar and Gračan, 2011). The colour and shape should then be compared to 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 (Ivar do Sul et al. 2011, Schuyler et al. 2014, Witherington, 2002). 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 sub-adult and adult turtles are not yet available for hatchlings, but technological advances mean they will most likely be available in the near future 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 3D 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 (López-Castro et al*.* 2013). A study by López-Castro et al*.* (2013) tentatively identified 6 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 distribution 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) suggests recording the abundance of epibionts as well as the presence and decomposition 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 by-catch 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 the majority of publications on turtle entanglement focus on the issue of ghost fishing by lost gear and few report entrapment in other forms marine debris, for example those originating from land-based sources (n=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 synthesise all relevant grey literature (for example, Balazs, 1984, 1985b) 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 (Özdilek 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 (Özdilek et al*.* 2006, Triessnig, 2012). Standardised 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 database 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 focussed 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 to 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, utilising 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 minimise 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 meso-cosm 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 impact 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 programmes 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. This review had 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, is severely lacking (Brown and Macfadyen, 2007). Open reporting of plastic-turtle interactions is encouraged and urge such observations to be submitted for peer-reviewed publication where ever possible. Furthermore, cooperation among scientists, industry, governments and the general public is urgently needed to confront this rapidly increasing form of pollution.

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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)	Campani *et al.*, 2013	2010-2011	31	71	29.0-73.0	X	✓	✓
		Adriatic sea (Croatia, Slovenia)	Lazar and Gračan, 2011	2001-2004	54	35.2	25.0-79.2	X	✓	✓
		Central Mediterranean (Sicily)	Russo *et al.*, 2003	1994-1998	44	15.9	unknown	na	na	na
		Central Mediterranean (Italy)	Casale *et al.*, 2008	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
	Atlantic ocean	South–western Atlantic (Brazil, Parabia)	Mascarenhas *et al.*, 2004	2004	1	100	66	X	X	✓

Olive ridley (*Lepidochelys olivacea*)		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 Study**	***n***	**CCL range**	**Pelagic Juvenile**	**Neritic Juvenile**	**Adult**	**Debris type**
Loggerhead (*Caretta caretta*)	Atlantic ocean	North–eastern (Boa Vista, Cape Verde Islands)	Lopez-Jurado *et al.*, 2003	2001	10	62.0-89.0	X	✓	✓	Fishing
		North–eastern (Terceira Island, Azores)	Barreiros and Raykov, 2014	2004 - 2008	3	37.3-64.1	X	✓	✓	Fishing/ Land-based
	Mediterranean Sea	Tyrrhenian sea (Island of Panarea, Sicily)	Bentivegna, 1995	1994	1	48.5	X	✓	X	Land-based
		Central Mediterranean (Italy)	Casale *et al.*, 2010	1980-2008	226	3.8-97.0	✓	✓	✓	Fishing/ Land-based
Green (*Chelonia mydas*)	Indian Ocean	North-eastern (Darwin, Australia)	Chatto, 1995	1994	1	35	X	✓	X	Fishing
		North-eastern (Australia)	Wilcox *et al.*, 2013	2005-2009	14	unknown	na	na	na	Fishing
Hawksbill (*Eretmochelys imbricata*)	Indian Ocean	North-eastern (Darwin, Australia)	Chatto, 1995	1994	1	32.5	X	✓	X	Fishing
		North-eastern (Australia)	Wilcox *et al.*, 2013	2005-2009	35	unknown	na	na	na	Fishing
Olive ridley (*Lepidochelys olivacea*)	Indian Ocean	North-eastern (McCluer Island, Australia)	Jensen *et al.*, 2013	unknown	44	unknown	na	na	na	Fishing
		North-eastern (Australia)	Wilcox *et al.*, 2013	2005-2009	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	1996-2011	18	2.01-80.0	X	✓	✓	Fishing
Flatback (*Natator depressus*)	Indian Ocean	North-eastern (Darwin, Australia)	Chatto, 1995	1994	1	25.5	X	✓	X	Land-based
		North-eastern (Australia)	Wilcox *et al.*, 2013	2005-2009	3	unknown	na	na	na	Fishing
Multiple	Indian Ocean	North-eastern (Australia)	Wilcox *et al.*, 2014	2005-2012	336	unknown	na	na	na	Fishing

*CCL = Curved Carapace Length

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

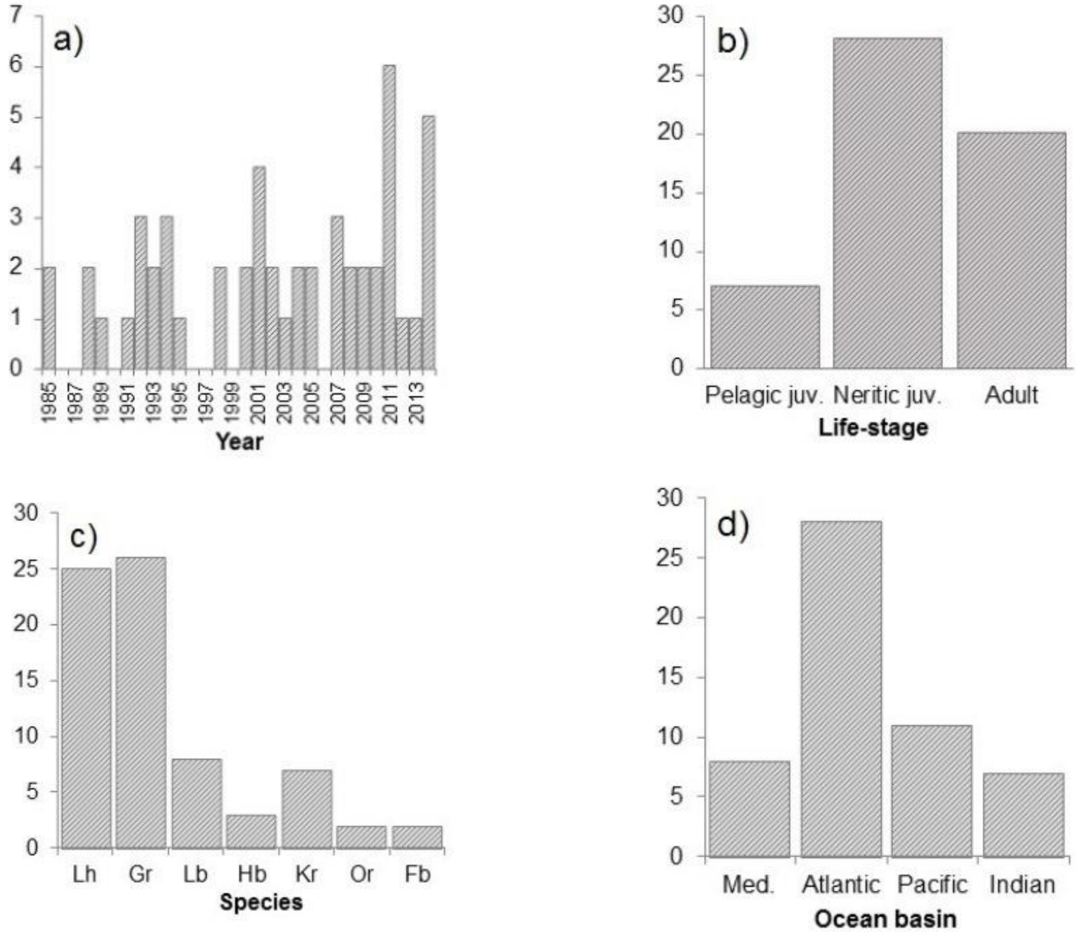

Figure 1. Number of publications returned from literature search per a) Year (between 1985 and 2014) b) Life-stage c) Species (Lh = Loggerhead, Gr = Green, Lb = Leatherback, Hb = Hawksbill, Kr = Kemp's ridley, Or = Olive ridley and Fb = Flatback), d) Ocean basin

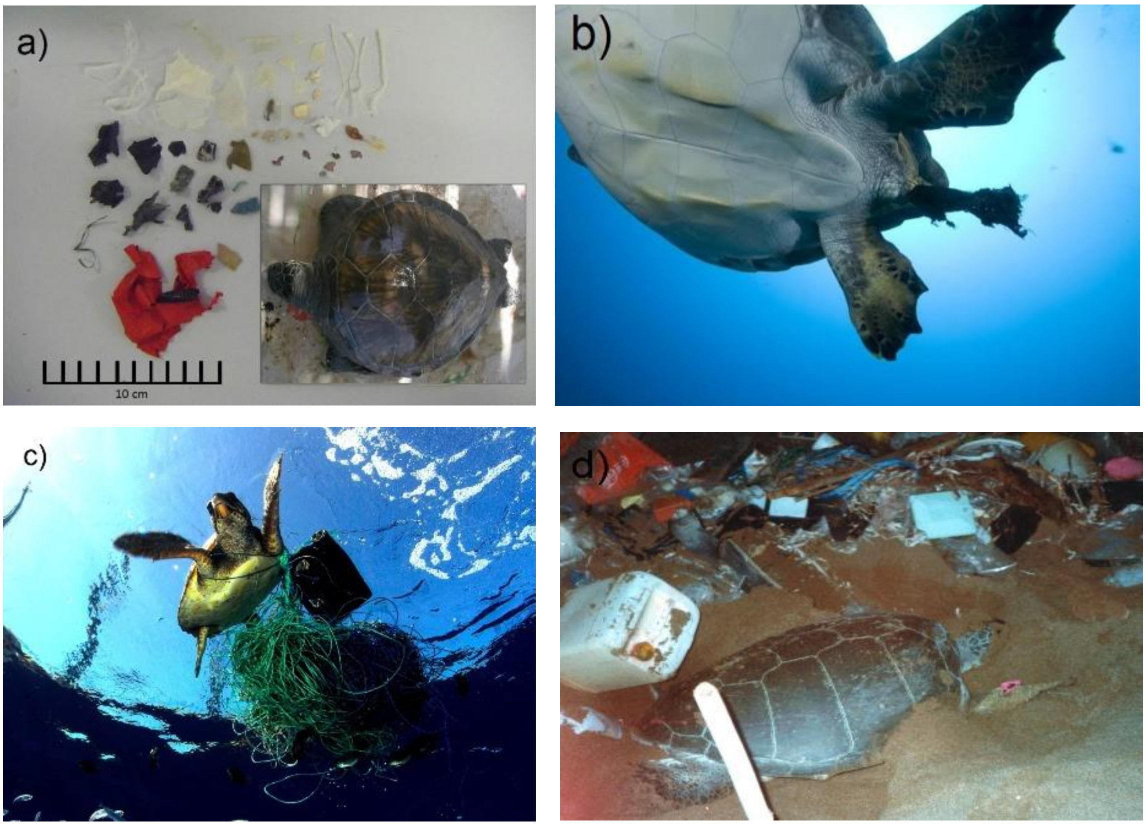

Figure 2. Plastics and marine turtles: a) Plastic fragments extracted from the digestive tract of a necropsied juvenile green turtle (inset), found stranded in northern Cyprus (Photo: Emily Duncan); b) Plastic extruding from a green turtle's cloaca in Cocos Island, Costa Rica. (Photo: Cristiano Paoli); c) Loggerhead turtle entangled in fishing gear in the Mediterranean Sea (north of Libya). (Photo: Greenpeace©/Carè©/Marine Photobank); d) Female green turtle attempting to nest amongst beach litter, northern Cyprus in 1992 prior to the commencement of annual beach cleaning. (Photo: Annette Broderick).

Chapter 3: A global review of marine turtle entanglement in anthropogenic debris: A baseline for further action

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Abstract

Entanglement in anthropogenic debris poses a threat to marine wildlife. Although this is recognised as a cause of marine turtle mortality, there remain quantitative knowledge gaps on entanglement rates and population implications. We provide a global summary of this issue in this taxon using a mixed methods approach, including a literature review and expert opinions from conservation scientists and practitioners worldwide. The literature review yielded 23 reports of marine turtle entanglement in anthropogenic debris, which included records for 6 species, in all ocean basins. Our experts reported the occurrence of marine turtles found entangled across all species, life stages and ocean basins, with suggestions of particular vulnerability in pelagic juvenile life stages. Numbers of stranded turtles encountered by our 106 respondents were in the thousands per year, with 5.5% of turtles encountered entangled; 90.6% of these dead. Of our experts questioned, 84% consider that this issue could be causing population level effects in some areas. Lost or discarded fishing materials, known as ‘ghost gear’, contributed to the majority of reported entanglements with debris from land-based sources in the distinct minority. Surveyed experts rated entanglement a greater threat to marine turtles than oil pollution, climate change and direct exploitation but less of a threat than plastic ingestion and fisheries bycatch. The challenges, research needs and priority actions facing marine turtle entanglement are discussed as pathways to begin to resolve and further understand the issue. Collaboration among stakeholder groups such as strandings networks, the fisheries sector and the scientific community will facilitate the development of mitigating actions.

Introduction

Marine plastic pollution

Anthropogenic materials, the majority of them plastic, are accumulating on the surface of the oceans, in the water column and on the seabed (Thompson et al. 2004). The durability of plastic means that it may persist for centuries (Barnes et al. 2009). It is estimated that 4.8 to 12.7 million tonnes of plastic waste could be entering the marine environment annually (Jambeck et al. 2015). Over 700 marine species have been demonstrated to interact with marine plastic pollution (Gall & Thompson 2015), which presents a risk to animals through ingestion, entanglement, degradation of key habitats and wider ecosystem effects (Nelms et al.2016). Megafauna such as marine turtles with complex life histories and highly mobile behaviour are particularly vulnerable to its impacts (Schuyler et al. 2014).

Entanglement in marine litter

Entanglement in plastic debris is recognised as a major risk for many marine species (Laist 1987,Vegter et al. 2014). This has become sufficiently high profile that the European Union's Marine Strategy Framework Directive (MSFD) Technical Subgroup on Marine Litter has announced that it will develop a dedicated monitoring protocol for its next report (MSFD GES Technical Subgroup on Marine Litter 2011). Entanglement has the potential to cause a range of fatal and non-fatal impacts such as serious wounds leading to maiming, amputation, increased drag, restricted movement or choking (Votier et al. 2011, Barreiros & Raykov 2014, Lawson et al. 2015).

Types of marine debris causing entanglement

The debris causing this entanglement falls into 2 broad categories. Firstly, hundreds of tons of fishing gear are lost, abandoned or discarded annually, forming ‘ghost gear’ which passively drifts over large distances, sometimes indiscriminately ‘fishing’ marine organisms (Macfadyen et al. 2009, Wilcox et al. 2013). This gear is commonly made of non-biodegradable synthetic material that will persist in the marine environment, potentially become biofouled by marine organisms and act as a fish aggregating device (FAD), attracting both grazers and predators such as marine turtles (Filmalter et al. 2013, Wilcox et al. 2013). It is important to distinguish here between ‘entanglement’ and ‘bycatch’. Bycatch can be defined as unselective catch of either unused or unmanaged species during fishing, with a particular focus on ‘active’ gear, whereas ghost gear can be defined as equipment of which the fisher has lost operational control (Smolowitz 1978, Davies et al. 2009). Therefore, here we consider animals caught in passive ghost fishing gear as entangled, not bycaught. Secondly, there have also been reports of entanglement in litter from land based sources (Chatto 1995, Bentivegna 1995, Santos et al. 2015). In this review we do not include bycaught turtle only those that have become entangled in passive anthropogenic debris such as ghost gear or land-based debris.

Current knowledge gaps regarding turtle entanglement

Despite turtle entanglement being recognised as one of the major sources of turtle mortality in northern Australia and the Mediterranean, there is a quantitative knowledge gap with respect to the entanglement rates and possible implications in terms of global populations (Casale et al. 2010, Wilcox et al. 2013, Camedda et al.

2014, Gilman et al. 2016). A recent literature review by Nelms et al. (2016) returned only 9 peer-reviewed publications on marine debris entanglement and turtles (Bentivegna 1995, Chatto 1995, López-Jurado et al. 2003, Casale et al. 2010, Santos et al. 2012, Jensen et al. 2013, Wilcox et al. 2013, 2015, Barreiros & Raykov 2014). Of these, 7 were focused on ghost fishing gear, highlighting the distinct lack of knowledge of entanglement in debris from landbased sources. Even fewer of these studies focused on the potential variable susceptibility among life stages or species, with only one paper, Santos et al. (2012), reporting that the majority of entangled olive ridley turtles *Lepidochelys olivacea* on the Brazilian islands of Fernando de Noronha and Atol das Rocas were sub-adults and adults.

Research rationale in terms of marine turtles and pollution

In terms of global research priorities for sea turtle conservation and management, understanding the impact of pollution is considered of high importance (Hamann et al. 2010, Rees et al. 2016). To evaluate this effectively, the impact of anthropogenic debris, specifically, must be considered at a species and population level. Additionally, it is important to understand the variation in entanglement rates among species and life stages to better evaluate vulnerability and the frequency of interactions with different debris types (Nelms et al. 2016). Once these have been established, opportunities for delivering effective education and awareness can be given or other mitigation planned (Vegter et al. 2014).

Here, we define marine turtle entanglement as ‘the process under which a marine turtle becomes entwined or trapped within anthropogenic materials.’We sought to include discarded fishing gear (ghost fishing) as well as land-based sources. The aim of this study was to (1) review existing, and obtain new, reports of the occurrence and global spatial distribution of marine turtle entanglement; (2) gain insights into patterns of species, life stage and debris type involved across entanglement cases; and (3) glean an insight into the change in prevalence of marine debris entanglement over time. To address these, a mixed methods approach was employed, involving a literature review and an elicitation of expert opinions. Given the difficulty of acquiring robust standardised data, this review is intended to highlight the value of mixed methods as a first step to understand complex conservation issues, and to provide suggestive yet relevant indications as to the scale of the threat of entanglement to marine turtles.

Materials and Methods

Literature review

In January 2016 and again in June 2017 (during the manuscript review process), all relevant literature was reviewed that may have contained records of marine turtle entanglement. ISI Web of Knowledge, Google Scholar and the Marine Turtle Newsletter (www.seaturtle.org) were searched for the terms ‘entanglement’, ‘entrapment’, ‘ensnare’ or ‘ghost fishing’ and ‘turtle’. The first 200 results were viewed, with results very rarely fulfilling the criteria after the first 20; spurious hits were ignored and all relevant references were recorded and investigated.

Elicitation of expert opinions

During the period 1–30 April 2016, an online questionnaire survey was conducted to investigate 3 main topics of interest: (1) the occurrence and global spatial distribution of sea turtle entanglement; (2) species, life stage and debris type involved; and (3) the change in entanglement prevalence over time. A total of 20 questions requiring both open and closed responses from a range of experts were used to obtain insight into the scale of marine turtle entanglement.

We clearly explained to the respondents the definition of ‘marine turtle entanglement’ specific to this study. Grid-like responses and Likert scales, offering potential answers from a range of ordinal options, were used to aid in achieving a quantitative assessment of the issues (Elaine & Seaman 2007) (see Box S1).

Potential participants for this questionnaire were identified from lead authorship of papers compiled in the recent review on the effects of marine plastic debris on turtles from Nelms et al. (2016), and our review due to their involvement in research into marine debris. From reviewing the few published reports, it was apparent that governmental stranding networks, sea turtle rescue and rehabilitation centres and conservation projects may also hold many unpublished records of entanglement occurrence. A comprehensive list of such organisations from seaturtle.org (www.seaturtle.org/groups/; accessed 24 March 2016) was used to find more expert contacts to participate in the questionnaire. Additionally, considering the aim of attaining an appropriate number of respondents while avoiding potential sampling biases due to researchers' personal networks and perceptions about the issue (Newing 2011), we employed respondent-driven sampling; this purposive sampling approach involves requesting those directly contacted to recruit additional participants among colleagues, peers and other organisations that may have knowledge of additional records of marine turtle entanglement.

From this first questionnaire, an initial report was produced and sent to the expert respondents (n = 106) to share the results and thoughts that arose from the first questionnaire. This included 8 initial figures produced from the data given by respondents in the original questionnaire to aid feedback of our results (these were draft versions of Figs. 2, 3 & 4). Following this, during the period 24 May to 30 June 2016, a followup questionnaire survey was conducted with the expert participants of the first questionnaire survey who were then invited to comment and answer 10 open and closed questions (see Box S2) This aimed to further understand the challenges, future requirements (both research and priority actions) and perceptions of the likelihood of population level effects of marine turtle entanglement. In this second questionnaire, respondents were asked to comment on our initial results and to provide suggestions on future knowledge gains and actions. Their answers were categorised using an inductive approach; summary themes were identified through the process of directly examining the data (Elo & Kyngäs 2008), instead of having predefined categories.

Results

Literature review

Our literature search yielded 23 reports regarding entanglement in multiple species of marine turtles, the majority of which were peer-reviewed publications (n = 17) with additional grey literature reports (n = 6). Species included loggerhead *Caretta caretta* (n = 7), green *Chelonia mydas* (n = 7), leatherback *Dermochelys coriacea* (n = 5), hawksbill *Eretmochelys imbricata* (n = 5), olive ridley *Lepidochelys olivacea* (n = 9) and flatback *Natator depressus* (n = 2). There were no records for Kemp's ridley *Lepidochelys kempii* (Table 1). Of these publications, 18 reported entanglement due to ghost fishing or fisheries materials and 7 recorded entanglement in landbased plastic debris; 7 publications reported the size range and life stage of the entangled turtles. These publications highlighted a range of impacts of entanglement, such as serious wounds leading to maiming, amputation or death, increased drag, restricted movement or choking that were further illustrated by photographs from collaborating experts (Figure 1).

Elicitation of expert opinions

Survey response rates and demographics

From an estimated pool of ca. 500 potential contacts, the 'Marine Turtle Entanglement Questionnaire' was received and completed by a total of 106 expert respondents from 43 countries. However, due to the anonymous nature of the survey and the potential augmentation from the use of respondent-driven sampling, it is not possible to determine how many of those initially contacted took part in the survey.

All ocean basins were covered; the respondents' main oceanic region of work was given as: Atlantic (34.8%;n = 39), Pacific (18.9%; n = 20), Caribbean (25.5%; n = 27), Mediterranean (9.4%; n = 10) and Indian (9.4%; n = 10). Respondents experienced a wide range in the number of annual stranding cases in their respective study sites (annual maxima given in the survey; mean ± SE = 239.9 ± 71.7, range = 0 to 4100, n = 97) but in total, through addition of the respondents' answers, they are responsible for attending an estimated 23 000 stranded turtles yr−1. Respondents also generally had many years of experience dealing with and reporting marine turtle strandings (range = 2 to 42 yr, mean ± SE = 15.6 ± 1.1, n = 98), confirming them as having relevant experience to answer the survey. The second follow-up questionnaire sent to all respondents (n = 106) received 63 responses with respondents from 31 countries.

Rates of entanglement

A majority of respondents (84.3%; n = 101) had encountered cases in which turtles were entangled in anthropogenic debris. When broken down by species, the proportion of stranded turtles that were entangled did not differ significantly (Kruskal-Wallis: $X^2 = 4.59$, df = 6, p = 0.59) (Figure 2a). There was a low percentage incidence for all species, with the grand median rate of 5.5%, although there was considerable inter- and intraspecific variation, with incidences in different responses ranging from 0 to 95.5%. In terms of the proportion of marine turtles alive when found entangled, there were significant interspecific differences (Kruskal-Wallis: $X^2 = 19.62$, df = 6, p =0.003). The proportion found alive (grand median =9.4%) was significantly higher in green (25.5%) and loggerhead (15.5%) turtles than in all other species (5.5%) (Figure 2b).

Entanglement rates also differed amongst life stages for each species. Whilst respondents indicated that all life stages of each species had been affected by entanglement, the results suggested adults were most impacted in leatherback and olive ridley turtles, whereas for the remaining species respondents indicated a higher rate of entanglement in juveniles (pelagic and neritic; Figure 3).

When considering this issue over time (over the last10 yr), a similar proportion of respondents (35.8% of 106) thought the prevalence of entanglement had increased or remained the same, while the remainder thought it had decreased (8.5%) or were unsure (19.8%). Among those respondents that noted an increase, some (n = 4) suggested that this may be caused by an increase in reporting and awareness, while others (n = 9) indicated the development of coastal fishing activities might be a factor. When asked to consider a shorter time period (the last 5 yr), the majority of respondents believed that the prevalence of entanglement they had experienced had remained stable (51.9%), whilst the others thought it had increased (29.2%), decreased (3.8%) or were not sure (15.1%).

Entanglement materials

The majority of entanglements recorded were with lost/discarded fishing gear (Figure 4). A clear distinction was made between ‘active’ and ‘lost/discarded’ fishing gear to try and separate incidents due to bycatch and subsequent stranding from those caused by ghost fishing. The number of responses on the occurrence of ghost fishing (GF) through discarded fishing debris (rope, net and line) was generally slightly higher than for bycatch (BC) through active gear.

A smaller percentage of respondents specified cases of turtle entanglement in land-based sources, from polythene sheeting (n = 71), woven sacks (n =72) and non-fishing rope/twine (n = 68). But in only a few incidences were these said to be common occurrences (polythene sheeting [n = 3], woven sacks [n = 4], non-fishing rope/twine [n = 7]). Respondents were asked to comment on the occurrence of ‘other’ entangling materials (n = 54) and to provide examples (n = 20) that caused turtle entanglement. This included debris from land-based sources (plastic -balloon string, canned drink ‘6-pack’ rings, kite string, plastic chairs, plastic packaging straps, wooden crates and weather balloons) and debris from other maritime activities (boating mooring line, anchor line and discarded seismic cable).

Scale of issue

In order to obtain further insights into the potential scale of this issue, respondents to the second survey were asked whether they thought entanglement in anthropogenic debris is causing population-level effect in marine turtles. Of the 63 respondents, 84.1% thought that this was probable, very likely or definite (Figure A1). There was no significant difference in scaled responses by ocean basin (Kruskal-Wallis:X^2 = 1.82, df = 4, p = 0.77). In order to assess the relative importance of different threats according to experts, we also sought the experts’ opinions on how they thought entanglement in anthropogenic debris compared to other threats to marine turtles (i.e. ‘plastic ingestion’, ‘oil pollution’, ‘fisheries bycatch’, ‘direct exploitation’ and ‘climate change’). Although between 6.35 and 25.4% were unsure, there was a strong opinion that plastic ingestion and fisheries bycatch were greater threats, and that oil pollution, climate change and direct exploitation were less severe threats than entanglement (Figure 5).

Challenges, priority actions and research needs

Respondents to the second survey converged on a limited number of themes when considering the challenges, research needs and priority actions within marine turtle entanglement. The challenges to addressing the issue (115 suggestions) could be grouped into 5 major categories: law and enforcement (23.5%; n = 27); sources and spatial extent of entanglement materials (24.3%; n = 28); education and innovation (24.3%; n = 28); understanding the full extent of the threat (18.3%; n = 21); and human response to entangled turtles (9.6%; n = 11) (Table 2). Seven major research areas were suggested by respondents (91 suggestions): more specific reporting and monitoring or a common database (23.1%; n =21); mapping the threat/spatio-temporal hotspots (31.9%; n = 29); identifying entanglement materials and sea turtle interactions (24.2%; n = 22); understanding post-release mortality and physical effects (3.3%; n = 3); socio-economic impacts (4.4%; n = 4); innovation of new replacement materials (6.6%; n =6); and demographic risk assessments (6.6%; n = 6) (Table 3). Priority actions (n = 121 suggestions) that respondents believe would help reduce turtle entanglement were grouped into 5 major areas: education/ stakeholder engagement (31.4%; n = 38); fisheries management and monitoring (26.4%; n = 32); research (5%; n = 6); law and enforcement (20.7%; n = 25); and development of alternative materials and methods (16.5%; n = 20) (Table 4).

Discussion

Global distribution

Our review and elicitation of expert opinions demonstrate that marine turtle entanglement is an issue operating at a global scale, occurring in all species, throughout their geographic range. We sought to answer key knowledge gaps surrounding the issue of turtle entanglement in marine debris as previously highlighted by Vegter et al. (2014) and Nelms et al. (2016). Difficulties in investigating these knowledge gaps are in part due to a lack of robust data. This highlights the importance of using mixed methods to access expert opinion to gain an insight into this global threat. The growing use of expert knowledge in conservation is driven by the need to identify and characterise issues under limited resource availability, and the urgency of conservation decisions (Martin et al. 2012).

Acknowledging the incomplete coverage of our estimates, given the mean estimated number of strandings and mortality rates, in the order of 1000 turtles die annually as a result of entanglement in the areas monitored by our respondents. These levels are likely a profound underestimation of the scale of this issue as the coverage of these actors is far from comprehensive. Second, it is well known that not all dead turtles strand (Epperly et al. 1996, Sasso & Epperly 2007), especially small and pelagic animals, and there can also be decay of entangled animals. Additionally, some of our respondents commented that detection of stranded animals may be further confounded due to take of stranded animals for human consumption.

Species differences

Although there was no interspecific difference in the incidence of entanglement, most peer-reviewed publications featured olive ridley turtles, with some experts reporting high incidences of entanglement for this species. Stelfox et al. (2016) noted that olive ridley turtles accounted for the majority of sea turtles identified as entangled (68%; n = 303), and this could be for the following reasons. Firstly, this species, which often exhibits mass nesting in the hundreds of thousands of individuals, is highly numerous, and at particularly high densities in some areas, leading to entanglement hotspots (Jensen et al. 2006, Koch et al. 2006, Wallace et al. 2010a). Secondly, the olive ridley forages along major oceanic fronts which are known to aggregate marine debris (Polovina et al. 2004, McMahon et al. 2007). Finally, their generalist feeding behaviour potentially attracts them to feed opportunistically on biofouled marine debris such as ghost gear (Stelfox et al. 2016).

Life stages

Entanglement was reported to occur in all life stages (pelagic juveniles, neritic juveniles and adults) across all species (the exception being flatback turtles which have no pelagic juveniles; Hamann et al. 2011). Perhaps of greatest concern is the signal of high entanglement incidence in the pelagic juvenile stage: despite the general inaccessibility of sampling this life stage, they are still appearing as stranded entangled. The currents that transport hatchlings to oceanic convergence zones are also now recognised as concentrating floating anthropogenic debris, creating the capacity for an ecological trap for these young turtles, whether it be through ingestion or entanglement (Nelms et al. 2016, Ryan et al. 2016). Many respondents considered that entanglement could be having a population level effect; a distinct possibility if this there is a large impact on this cryptic life stage and on pelagic foraging adults (Mazaris et al. 2005).

Entangling materials

Respondent data highlighted that the majority of entanglements were the result of fishery-based material and other maritime activities. The issue of ghost fishing featured highly, with numerous responses reporting entanglement within lost/discarded gear. This gear is often lost, abandoned or discarded when it becomes derelict, attracting scavengers and acting as FADs (Gilman 2011). Subsequently, species such as marine turtles become entangled within the gear, perhaps encouraged by this process of ‘selfbaiting’ (Matsuoka et al. 2005).

Change in fishing practice

The issue of ghost fishing appears to have worsened since the 1950s, as the world’s fishing industries have replaced their gear, which was originally made of natural fibres such as cotton, jute and hemp, with synthetic plastic materials such as nylon, polyethylene and polypropylene. Manufactured to be resistant to degradation in water means that once lost, it can remain in the marine environment for decades (Good et al. 2010). Furthermore, there has also been a shift in the type of synthetic nets being selected; for example, fishers in part of Southeast Asia now increasingly favour superfine nets. Although this can help increase catches, the twine thinness means that they break easily and are difficult to repair once damaged (Stelfox et al. 2016). The incidences of entanglement caused by this form of pollution in our expert surveys indicates that this source of mortality for marine turtles mirrors that in marine mammals and sea birds, which has increased substantially over the last century(Tasker et al. 2000, Good et al. 2010, McIntosh et al. 2015).

Differentiation from bycatch

It is quite plausible that ghost fishing may be working synergistically alongside bycatch, but because of its more cryptic nature this means that understanding its role in marine turtle mortality is much more difficult. Bycatch is better understood. For example, the analysis of catch rates in the Mediterranean allowed for the estimation of 132 000 captures and 44 000 incidental deaths per year (Casale 2011). Likewise, cumulative analysis of catch rates in US fisheries estimated a total of 71 000 annual deaths prior to the establishment of bycatch mitigation methods. Since these measures were implemented, mortality estimates are ~94% lower (4600 deaths yr−1) (Finkbeiner et al. 2011). This highlights the importance of informed estimates to monitor the success of mitigation methods. In addition to bycatch mortality estimates, spatial and temporal patterns of bycatch incidences can be identified. Using onboard observer data, Gardner et al. (2008) found seasonal changes in catch distributions of loggerhead and leatherback turtles in the North Atlantic, with patterns of spatial clustering from July to October. Analysed on a global scale, Wallace et al. (2010b) were able to highlight region− gear combinations requiring urgent action such as gillnets, longlines and trawls in the Mediterranean Sea and eastern Pacific Ocean. Generating such estimates of catch rates and spatial/temporal patterns for entanglement are not yet possible due to the lack of quantitative information.

Land-based plastic entanglements

The domination of fisheries-based materials in the results does not mean that land-based plastics are not a source of entanglement. The increased input of plastic debris from terrestrial run-off means that these interactions are only likely to increase (Jambeck et al. 2015). Our literature search and ‘other’ materials stated by respondents contained a variety of items causing entanglement that could be decreased by reduction of use, replacement with more degradable alternatives and better waste management and recycling. The prevalence of these materials in the marine environment will very much depend on future waste governance, especially in those countries that generate the most plastic waste (Jambeck et al. 2015). A future technological solution which is currently being investigated or adopted such as Thailand and India is the pyrolysis of plastics. This process produces fuel from waste plastic, a better alternative to landfill and a partial replacement of depleting fossil fuels (Wong et al. 2015).

Caveats

It is important to recognise the biases associated with using stranding animals for data collection. Within and between stranding sites there are differences in turtle foraging ecology, life stages and proximity to human habitation (Bolten 2003, Rees et al. 2010), and therefore they are exposed to different levels and types of potential entangling materials. Individual turtles therefore may not represent a homogeneous group in terms of entanglement occurrence within that population (Casale et al. 2016). Additionally, recovered carcasses represent an unknown fraction of at-sea mortalities, with physical oceanography (e.g. currents) and biological factors (e.g. decomposition) affecting the probability and location of carcass strandings (Hart et al. 2006). However, examining reports of stranded animals represents a vital opportunity for research and can provide insights into the impacts of anthropogenic threats which would otherwise go undetected (Chaloupka et al. 2008, Casale et al. 2010). In addition, stranding information aids with the assessment of harder-to-access life stages, yielding key information on the risk to specific resident populations and contributing to building a worldwide perspective for conservation issues (Chaloupka et al. 2008, Casale et al. 2016). Indeed, this was the aim of our study: using stranding data from expert respondents to gain an initial indication of the estimated magnitude of this threat.

Surveying experts can be a powerful tool for obtaining insights on particular topics not widely known by others (Martin et al. 2012). Expert knowledge and opinions may be the result of training, research, skills and personal experience (Burgman et al. 2011a). In this study, we sought the opinions of conservation scientists and practitioners with experience in marine turtle entanglement and strandings. Due to the purposive sampling nature of our approach, we aimed to identify people with relevant experiences instead of focusing on obtaining a random selection of representatives; this is a widely used practice when undertaking social surveys that focus on particular subgroups or specialists (Newing 2011). Nevertheless, expert knowledge and opinions are also known to be subject to biases, including overconfidence, accessibility and motivation (see e.g. Burgman et al. 2011b and Martin et al. 2012). In the absence of empirical data to validate our findings, this remains as simply suggestive but nevertheless relevant information in terms of identifying a potentially important conservation issue and providing relative indications of the scale of entanglement as a threat to sea turtles.

Future actions and recommendations

Ghost fishing

Issue and policy. Presently, a large knowledge gap exists regarding effects of ghost fishing. While there has been some progress in documenting the frequency of loss from passive gear such as gillnets, little is known about loss from active gears; effective methodology to estimate the persistence of types of gear such as trawl nets has yet to be developed (Gilman et al. 2013). While it would be optimal to switch all gear to more biodegradable materials, synthetic materials will continue to be used within fisheries for the foreseeable future. This is an issue that has been highlighted in policy by the Food and Agriculture Organization (FAO), who recommend the identification, quantification and reduction of mortality caused by ghost fishing by implementing this into fisheries management plans, increasing scientific information and developing mitigation strategies; but this appears still to be in its infancy (Gilman et al. 2013). This is also reflected in mandates within the International Maritime Organisation (IMO) and International Convention for Prevention of Pollution from Ships (MARPOL Annex V) (Stelfox et al. 2016).

Need for a global database and spatial hotspot identification. Undoubtedly a common global metadatabase recording the spatial distribution and abundance of possible entangling ghost gear as well as incidences of marine turtle entanglement incorporating a unit of effort metric would assist in quantifying the mortality due to ghost gear that is needed to inform policy (Nelms et al. 2016). A recent global review (dominated by the Atlantic and Pacific oceans) on marine megafauna by Stelfox et al. (2016) reported a total of 5400 individuals of 40 species that had been associated with ghost gear between 1997and 2015. They suggested this was a great underestimate due to lack of capacity to record incidence. Such data could feed into one of the major research priorities emphasised by respondents; modelling spatio-temporal hotspots of entanglement. An innovative study by Wilcox et al. (2013) used beach clean data and models of ocean drift to map the spatial degree of threat posed by ghost nets for marine turtles in northern Australia and map areas of high risk. With the input of more specific marine location data on ghost gear and the advocacy of the use of ever improving modelling, this could provide a powerful tool in the future.

Education and stakeholder engagement

Local initiative to reduce debris causing entanglement. On a more local and regional scale, many initiatives are being brought into place to encourage a reduction in the amount of ghost gear/plastic debris entering the ocean and combat discarding at sea by working closely with community education and engagement; another highlighted topic by our respondents. There are numerous examples: the sea turtle conservation program in Bonaire has started a ‘Fishing Line Project’ (www.bonaireturtles. org/ wpp/what-we-do/fishing-line-project) working with volunteers to train them on how to remove discarded line and nets from coral reefs, and the Zoological Society of London’s ‘Net-works’ (www.net-works.com) initiative has established a supply chain for discarded fishing nets from artisanal fishing communities in the Philippines to a carpet manufacturing company. With further replication of such community-based projects and stakeholder engagement, especially with artisanal fisheries awareness, the potential exists to start targeting hotspots of marine vertebrate entanglement directly.

Stranding networks training. Another set of stakeholders which will be important to engage are stranding networks. Responses to entangled turtles can often be slow, and respondents commented that many are not trained in the correct protocols to safely remove entangling materials. If stranding networks were fully trained in a standardised protocol for removal, the techniques could then be passed on through educational training programmes to the fishing community, quickening the response to such incidences. This is already beginning to happen for bycatch cases; Sicilian fisherman now actively volunteer to take part in the rescue of turtles in difficulty and are trained in contacting the competent authorities for the transfer of turtles to the nearest recovery centres. This level of involvement by workers in the fishery sector was stressed and encouraged through both effective education activity and specific targeted study campaigns (Russo et al. 2014).

Future research avenues into marine turtle entanglement

Respondents raised the issue of post-release mortality and the importance of behavioural research into the interactions between marine turtles and potential entangling materials present in the marine environment. The prominence of this has been emphasised within other taxa; for example, postrelease mortality can result from long-term chronic effects of injuries in pinnipeds even after the entanglement has been removed (McIntosh et al. 2015). Furthermore, it has been argued that some colonial seabirds released from entangling plastic would not survive without human intervention (Votier et al. 2011).

To validate the success of release protocols after entanglement incidents (as mentioned above), techniques could be employed from other areas of marine turtle research. Satellite telemetry has already been used in a multitude of ways to provide information on conservation issues facing marine turtles; a number of studies have used this technique to consider post-release mortality after bycatch fisheries interactions (reviewed in Jeffers & Godley 2016). Deploying tagged turtles that have been involved in entanglements could aid in the understanding of survival after these events as well as simultaneously providing information on the location of sea turtles, feeding into information on entanglement hotspots to target mitigation actions. The benefits of utilising such techniques have been illustrated in other endangered species facing entanglement, such as studying mortality of silky sharks *Carcharhinus falciformis* in the Indian Ocean; estimates derived from satellite tracking showed that mortality due to entanglement was 5 to 10 times that of known bycatch mortality and provided evidence for a call advising immediate management intervention (Filmalter et al. 2013).

Other research methods and ideas could be modified from the study of plastic debris ingestion by sea turtles. Studies are currently underway to understand the selective mechanisms that lead to ingestion of plastic pieces (Schuyler et al. 2014, Nelms et al. 2016). For instance, a study by Santos et al. (2016) used Thayer's law of countershading to assess differences in the conspicuousness of plastic debris to infer the likelihood that visual foragers (sea turtles) would detect and possibly ingest the plastic fragments. Similar studies could be conducted to comprehend the underlying behavioural and physiological mechanisms that influence turtles to approach potential entangling materials when encountering them within the marine environment.

Similarly, comprehending how important the level of biofouling on this synthetic debris is in contributing to the likelihood of entanglement will be important. Total fish catches by monofilament gillnets in Turkey was lower, as a result of accumulating detritus and biofouling increasing the visibility of the nets in the water column (Ayaz et al. 2006). Furthermore, the level of biofouling could indicate the age of ghost gear entangling marine turtles. Retrieved lost/discarded fishing gears are usually found fouled by macro-benthic organisms, so if a relationship between soak time and biofouling level could be established, these organisms could provide a valid methodology to age the gear and enable better estimates of ‘catches’ made by the respective net (Saldanha et al. 2003).

Finally, it will be important to undertake demographic studies, calculating rates of entanglement, especially for specific populations that are known to be particularly vulnerable to a combination of other anthropogenic threats. For species such as pinnipeds, which are less elusive (hauling out on land) than marine turtles, the literature describes different methods. For example, a proportion derived from account of entangled individuals from a sub-sample or an estimate of the total population (Raum-Suryan et al. 2009, McIntosh et al. 2015), or more recently, the use of mixed-effects models to obtain a prediction of the total number of seals entangled per year, by examining changes in entanglement rates over time and the potential drivers of these detected trends (McIntosh et al. 2015). However, this can only be achieved if reporting and recording such incidences in marine turtles improves in efficacy and standardisation.

Conclusions

Further research may show that the issue is more one of animal welfare than of substantive conservation concern to many marine turtle populations. It is clear, however, that entanglement with anthropogenic plastic materials such as discarded fishing gear and land-based sources is an under-reported and under-researched threat to marine turtles. Collaboration among stakeholder groups such as strandings networks, fisheries and the scientific community will aid in providing mitigating actions by targeting the issue of ghost fishing, engaging in education and producing urgently needed research to fill knowledge gaps.

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Species	Ocean basin	Study area	Reference	Year of Study	N	CCL range	Pelagic juvenile	Neritic juvenile	Adult	Debris type
Loggerhead	Atlantic Ocean	North–eastern (Boa Vista, Cape Verde Islands)	López-Jurado *et al.* (2003)	2001	10	62.0-89.0	X	✓	✓	Fishing
		North–eastern (Terceira Island, Azores)	Barreiros & Raykov (2014)	2004 -2008	3	37.3-64.1	X	✓	✓	Fishing/Land-based
	Mediterranean Sea	Tyrrhenian sea (Island of Panarea, Sicily)	Bentivenga (1995)	1994	1	48.5	X	✓	X	Land based
		Central Mediterranean (Italy)	Casale *et al.* (2010)	1980-2008	226	3.8-97.0	✓	✓	✓	Fishing/Land-based
	Global		Balazs (1985)	1967-1984	5	unknown	✓	✓	✓	Fishing
Green	Indian Ocean	North (Maldives)	Stelfox & Hudgins (2015)	2013-2015	2	unknown	na	na	na	Fishing
		North-eastern (Darwin, Australia)	Chatto *et al.* (1995)	1994	1	35	X	✓	X	Fishing
		North-eastern (Australia)	Wilcox *et al.* (2013)	2005-2009	14	unknown	na	na	na	Fishing
	Global		Balazs (1985)	1967-1984	24	unknown	✓	✓	✓	Fishing (21)/Land-based (3)
	Pacific Ocean	Central (Hawaii)	Francke *et al.* (2014)	2013-2014	51	unknown	✓	✓	✓	Fishing
			Chaloupka *et al.* (2008)	1982-2003	43	20.0-100.0	✓	✓	✓	Fishing
	Caribbean Sea	North-western (Venezuela)	Barrios-Garrido *et al.* (2013)	2013	1	unknown	na	na	na	Fishing
Leatherback	Indian Ocean	North (Maldives)	Stelfox & Hudgins (2015)	2013-2015	1	unknown	na	na	na	Fishing
	Pacific Ocean	North-western (USA)	Moore *et al.* (2009)	2001-2005	1	unknown	na	na	na	Fishing
	Global		Balazs (1985)	1967-1984	5	unknown	X	X	✓	Fishing
Hawksbill	Indian Ocean	North (Maldives)	Stelfox & Hudgins (2015)	2013-2015	6	unknown	X	✓	X	Fishing
		North-eastern (Darwin, Australia)	Chatto *et al.* (1995)	1994	1	32.5	X	✓	X	Fishing
		North-eastern (Australia)	Wilcox *et al.* (2013)	2005-2009	35	unknown	na	na	na	Fishing
	Global		Balazs (1985)	1967-1984	9	unknown	✓	✓	✓	Fishing (8)/Land based (1)
Olive ridley	Indian Ocean	North (Maldives)	Anderson *et al.* (2009)	1998-2007	25	10.0-61.0	✓	✓	X	Fishing (22)/Land-based (3)
		North (Maldives)	Stelfox & Hudgins (2015)	2013-2015	163	unknown	✓	✓	✓	Fishing
		North-eastern (McCluer Island, Australia)	Jensen *et al.* (2013)	unknown	44	unknown	na	na	na	Fishing
		North-eastern (Australia)	Wilcox *et al.* (2013)	2005-2009	53	unknown	na	na	na	Fishing
		North-eastern (Darwin, Australia)	Chatto *et al.* (1995)	1994	2	64	X	X	✓	Fishing
		North-western (Seychelles)	Remie & Mortimer (2007)	2007	1	unknown	X	✓	X	Unspecified
	Atlantic Ocean	South-western (Brazil)	Santos *et al.* (2012)	1996-2011	18	2.01-80.0	X	✓	✓	Fishing

	Global		Balazs (1985)	1967-1984	7	unknown	✓	✓	✓	Fishing
	Pacific Ocean	Central (Hawaii)	Francke *et al.* (2014)	2013-2014	1	unknown	na	na	na	Fishing
Flatback	Indian Ocean	North-eastern (Darwin, Australia)	Chatto *et al*. (1995)	1994	1	25.5	X	✓	X	Land-based
		North-eastern (Australia)	Wilcox *et al.* (2013)	2005-2009	3	unknown	na	na	na	Fishing
Multiple	Indian Ocean	North-eastern (Australia)	Wilcox *et al*. (2014)	2005-2012	336	unknown	na	na	na	Fishing
	Pacific Ocean	South-western (Australia)	Meager & Limpus (2012)	2011	5	unknown	na	na	na	Fishing

CCL, curved carapace length

Table 1. Summary of all studies on entanglement of marine turtles in plastic debris.

Major Challenges

Challenge Category	% of suggestions (n=115)	Challenges described	Direct quotes from respondents
Law & Enforcement	23.5	Management of both of industrial and small-scale artisanal fisheries The issue of discarded fishing gear at sea Ineffectiveness of Marine Protected Areas	*"Under-resourced fisheries management of small-scale fisheries"* *"Trawlers should file a report anytime they lose netting"* *"Shifting climate may render Marine Protected Areas as ineffective"*
Source of entanglement materials and Extent of current materials	24.3	Estimating the amount and durability of entangling material entering the sea Retrieving lost fishing gear Lack of accountability	*"Entangling material tends to be durable, so even if management scheme is put into place, have to deal with historic material already in the ocean"* *"In my region, lost/discarded fishing lines are a big issue"* *"Inability to determine source of entanglement debris (no accountability)"*
Education & Innovation	24.3	Fisherman education and awareness Developing a discipline to avoid abandoning fishing gear Sourcing alternative materials	*" Engagement/education/enticement to bring artisanal fishers in developing countries to a want to reduce turtle mortality"* *"Figuring out how to reach out to boaters/ fishermen with making them want to support sea turtle friendly habits"* *"Addressing amateur/recreational fishers is really hard. In my opinion, most of the discarded fishing lines are left by this group"* *"Creation of degradable nylon"*
Understanding the full extent of the threat	18.3	Lack of stranding networks ability to measure the impact of this in multiple areas Difficulty in determining if entanglement occurred pre- or post- mortem Survivorship of turtle found entangled alive	*"It is hard to estimate the total amount of entangled turtles, since these animals are highly migratory and tend to be scattered over wide areas. Additionally turtles that become entangled may quickly die and be predated. Scavengers, predators, wind and currents may prevent carcasses from coming ashore"* *"Most entanglement records rely on land-based sampling and stranding do not represent total deaths at sea"* *"It is hard to distinguish marine debris from active and ghost fishing gears"* *"Difficulty in determining if entanglement occurred pre- or post-mortem (for some entanglement types, such as discarded nets/line)"* *"Limited post-release monitoring of live entangled turtles"*
Response to entangled turtles	9.6	Detangle permits Discovery times needs to be quick	*"Very few people are trained and permitted to disentangle them"* *"Discovering entangled turtles quickly"* *"Entangled turtle can be challenging to disentangle especially if they are not anchored and instead are free swimming"*

	Ineffectiveness of reporting systems	*"Having a good system in place that stranding will be reported (people that see an entangled turtle have to be able to notify the correct organization) having a good system in place that stranding will be reported (people that see an entangled turtle have to be able to notify the correct organization)"*
	Lack of rehabilitation resources for entanglement incidents	*"Lack of rehabilitation resources for turtles hurt in incidents of entanglement"*

Table 2. Summary of major challenges regarding marine turtle entanglement given by respondents

Research needs

Research Need category	% of suggestions (n=91)	Research needs described	
More specific reporting and monitoring / common database	23.1	The creation of a common database	*"A common database, long lasting surveys and a programme on a national base for monitoring of the state of debris in the sea"*
		An increase in specificity of reporting of entanglements cases	*"Better monitoring/reporting of entanglement cases by species, life stage, region"*
			"Establish a protocol for sea turtle strandings networks for identify entanglements and report these"
		Collaboration of resource users in the marine environment	*"More collaboration with resource users in the marine environment in respect to reporting cases of entanglement"*
			"Getting information from fishermen when turtles get entangled. Support to Fisheries Division who can provide accurate information on net damage from reports by fishermen. Only a small percentage of stranded turtles will wash up ...carcasses may become destroyed prior to reaching those coasts"
Mapping the threat/ spatio-temporal hotspots	31.9	Using stakeholder knowledge	*"Surveys to fishermen (industrial, artisanal and sport) to understand where and when they discard nets or lines and in water monitoring programs in coastal areas with high pressure of artisanal and sport fishing"*
		Identifying and mapping the entanglement rates due to different gear types and materials	*"Understanding where the event occurs, such as targeting if the problem is more from floating debris versus debris in water column"*
		Modelling /mapping patterns of debris distribution, patterns of marine turtle migrations and the characterization of fisheries distributions.	*"Understanding overlap between sea turtle habitats (e.g. nesting and feeding grounds) with areas of high debris concentration (e.g. convergence zones)"*
			"Spatio-temporal scales. Hotspots"
Entanglement materials and sea turtle interactions	24.2	Studying sea turtle and debris behaviour and their interactions	*"Behavioural (foraging or sheltering) traits in different turtle species or populations that may them more vulnerable to entanglement"*
			"Investigate the behavioural characteristics of the turtles that lead to their entrapment in fishing gear with a view to improving mitigation actions"
Post release mortality and survival/physical effects	3.3	Understanding true post-release mortality and morbidity	*"The effects of flipper amputations on survival"*
Socio-economic impact	4.4	Special focus on the fisher community	*"What are the opportunities and barriers to intervention?"*
Innovation of new replacement materials & methods	6.6	The innovation of biodegradable alternatives to commonly used plastic materials	"Alternative materials for fishing and other things/activities"
Demographic risk assessments	6.6	The development of demographic risk assessments for threatened populations of turtles	"Develop the appropriate population demographic models for marine turtles to allow for assessment/identification of those mortality factor that are not detrimental to maintaining robust non threatened population of turtle"

Table 3. Summary of research needs regarding marine turtle entanglement given by respondents

Priority Actions

Priority Actions category	% of suggestions (n=121)	Priority Actions described	
Education / Stakeholder engagement	31.4	Fisher involvement/ education	*"Develop questionnaire for fishermen for their recommendations on how it would be possible to reduce turtle entanglement"* *"Partnership with local fishermen to locate and remove abandoned or lost fishing gear (ghost gear). Financial incentives to return discarded gears to shore"*
		Community/ public awareness campaigns up on marine litter	*"Organizing campaigns with scuba divers to clean sea bottom from the man debris and ghost nets/discarded fishing lines"* *"Implement an environmental stewardship certificate system among ocean users and create a global open access database of entanglements to facilitate research efforts"*
Fisheries management and monitoring	26.4	The development of traceable gear	*"Developing/using traceable gear in combination with introducing a fining policy"* *"Increased collaborations with commercial fisherman and recreational fisherman to better understand their needs and the needs of the turtles....and how these can be combined"*
		Stricter regulations	
Research/ knowledge	5	The implementation of the research needs stated above**	"We cannot say before understanding the main reasons, main sources and main habitats or localities in which entanglement occurs"
Law and Enforcement on entanglement material	20.7	Banning at-sea disposal of entangling materials	*"Enforcement of laws banning at-sea disposal of entangling material"*
		Better waste management and increased recycling efforts	*"Reduction of manmade debris, better waste management, more biodegradable products"*
Development of alternative materials/methods	16.5	Development of alternative materials/ methods	*"Development of less environmentally persistent materials to be used in nets, fishing line, etc."*
		Shifting gear type/ increasing the use of biodegradable materials	*"Different strategies to different fishing gear; from the coastal sport fishermen to high seas industrial fishermen"* *"Introduce biodegradable chord into selected net fisheries with high loss to ghost nets"*

Table 4. Summary of priority actions regarding marine turtle entanglement given by respondents

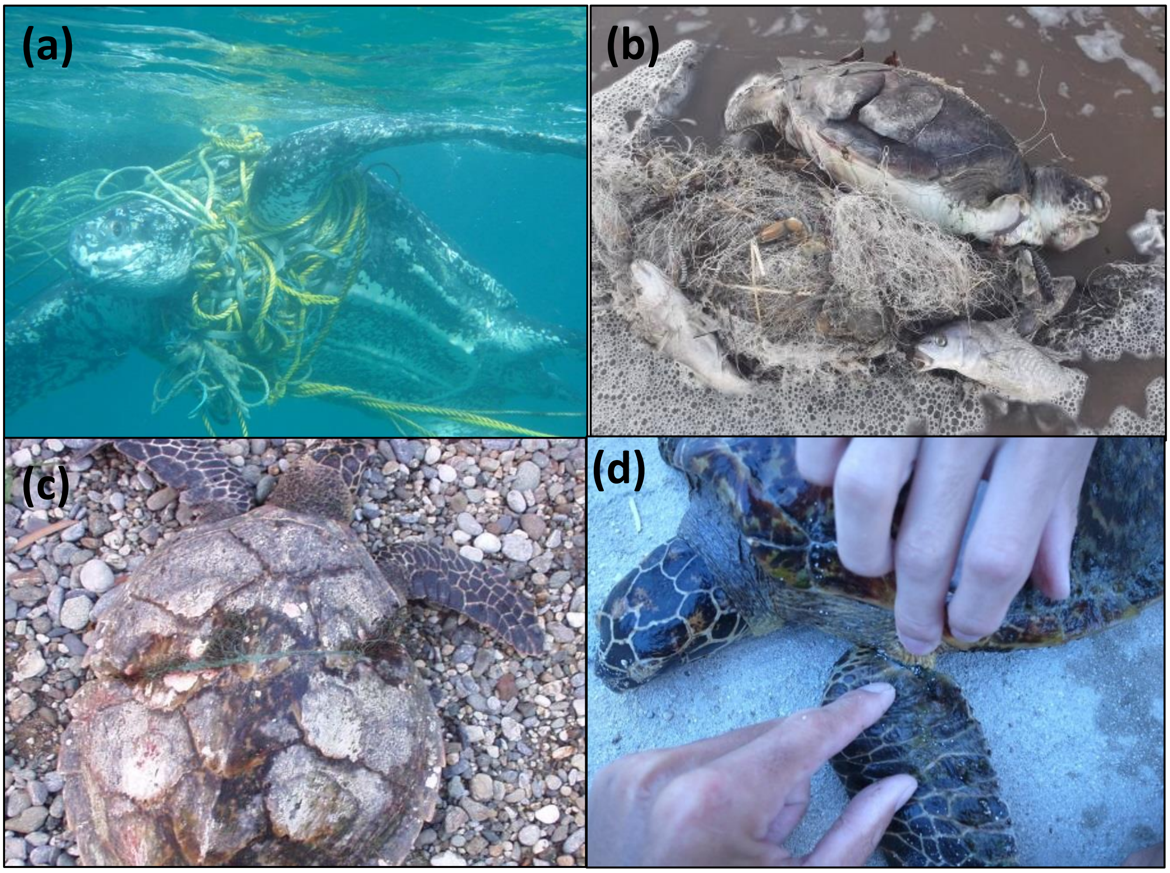

Figure 1. Impacts of marine turtle entanglement: (a) live leatherback turtle entangled in fishing ropes which increases drag, Grenada 2014 (photo: Kate Charles, Ocean Spirits); (b) drowned green turtle entangled in ghost nets in Uruguay (photo: Karumbe); (c) live hawksbill turtle entangled in fishing material constricting shell growth, Kaeyama Island, Japan 2001 (photo: Sea Turtle Association of Japan); (d) live hawksbill turtle with anthropogenic debris wrapped around front left flipper constricting usage of limb which could lead to amputation and infection, Kaeyama Island, Japan 2015 (photo: Sea Turtle Association of Japan). All photos used with express permission

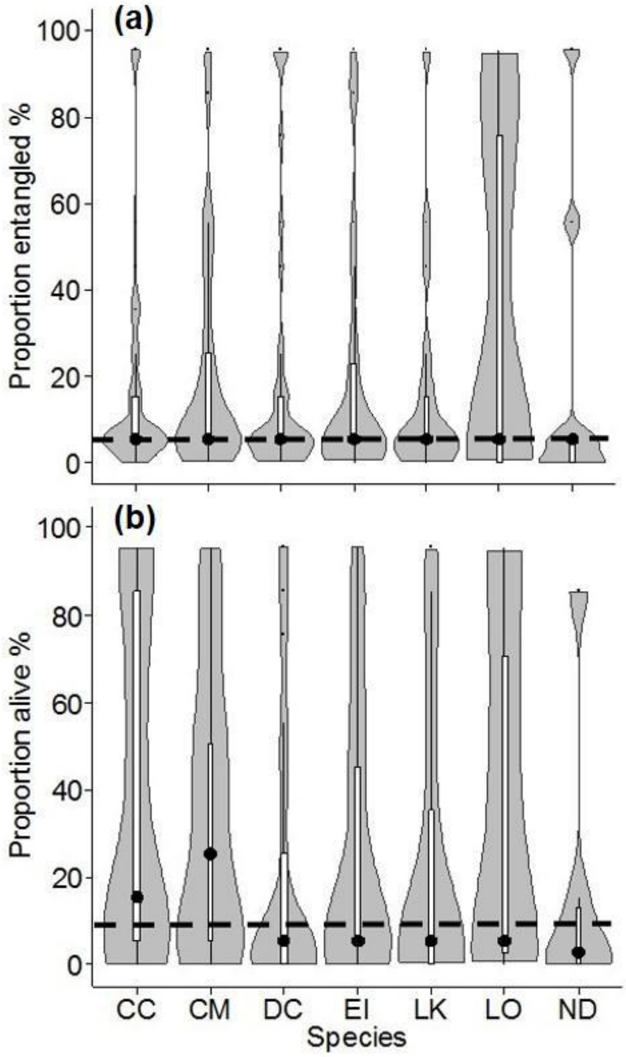

Figure 2. Inter-species comparison of the proportion of: (a) stranded individuals found entangled and (b) individuals found alive when discovered entangled. Violin plots show the kernel density of data at different values. Median (black dot) with interquartile range boxplot (black/white) and grand median (black dashed line). Turtle species abbreviations: CC: loggerhead *Caretta caretta*; CM: green *Chelonia mydas*; DC: leatherback *Dermochelys coriacea*; EI: hawksbill *Eretmochelys imbricata*; LK: Kemp’s ridley *Lepidochelys kempii*; LO: olive ridley *Lepidochelys olivacea*; ND: flatback turtle *Natator depressus*

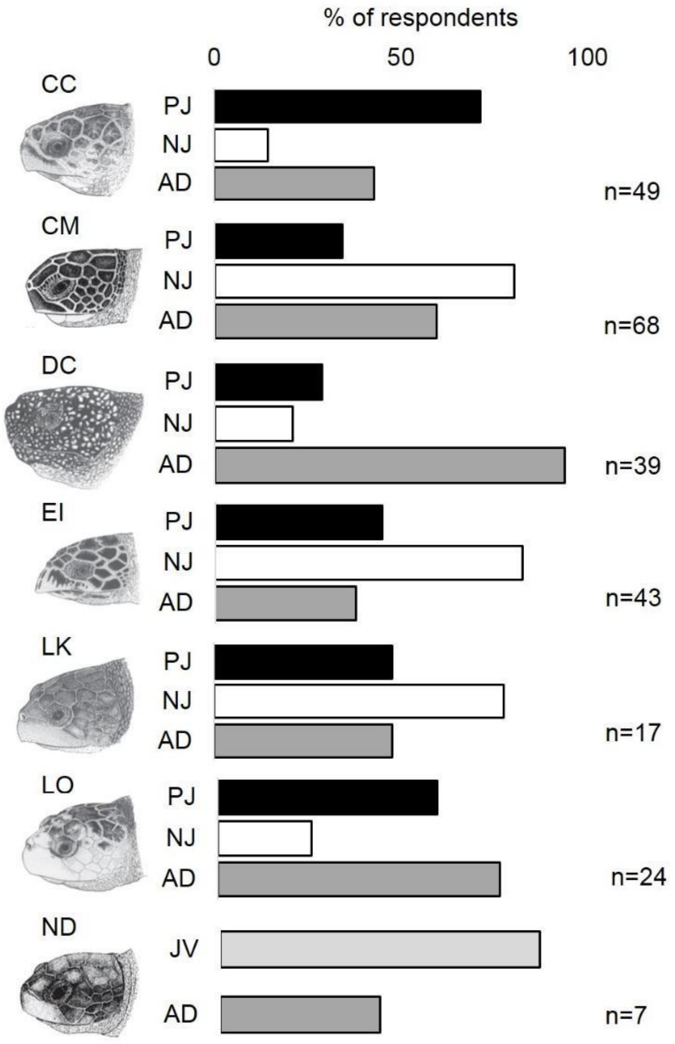

Figure 3. Inter-specific comparison of the breakdown of entangled sea turtle species by life stage. Black: pelagic juveniles (PJ); white: neritic juveniles (NJ); light grey: juveniles (JV); dark grey: adults (AD); see Fig. 2 for species abbreviations. Flatback turtles were only categorised into juvenile or adult classes with advice from species experts. Sea turtle skull figures used with permission of WIDECAST; original artwork by Tom McFarland

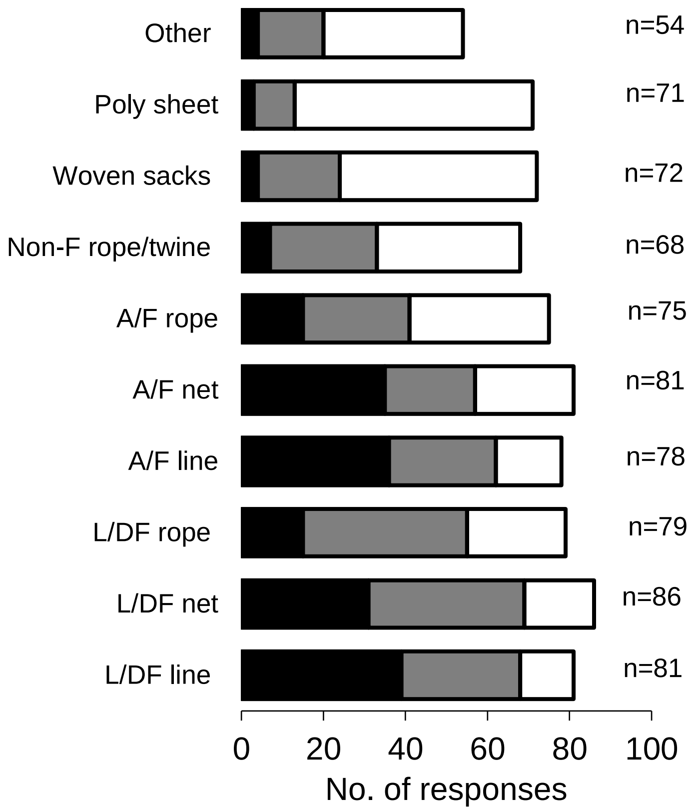

Figure 4. Entangling materials. L/DF: lost/discarded fishing; A/F: active fishing; Non-F: non fishing; Poly sheet: poly - ethylene sheeting. Black: common (10% or more of cases); grey: sometimes (less than 10% of cases); white: never. Not all participants categorised each material; total number of responses for each material shown on the right of the graph

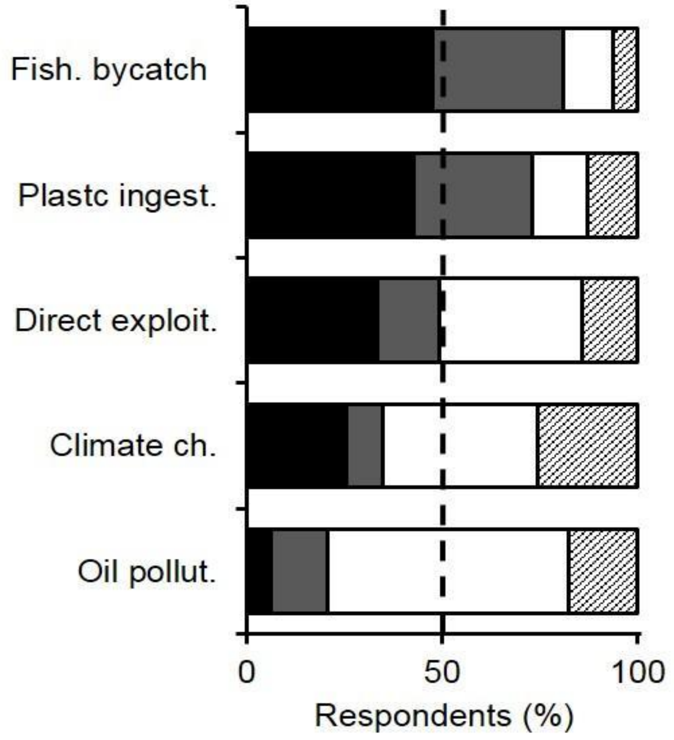

Figure 5. Responses to comparison of other threats faced by marine turtles compared to entanglement (n = 63). Black: greater than entanglement; grey: similar threat; white: less than entanglement; striped: unsure

Chapter 3: Supplementary Information

Box S1. First Questionnaire

Marine Turtle Entanglement Survey

Introduction & Background

Welcome to the survey of marine turtle entanglement in anthropogenic (man-made) debris. You are invited to take part in this study that aims to glean insight into the scale of this issue to ultimately aid in managing this threat. The study is being conducted by Emily Duncan, Zara Botterell and Prof. Brendan Godley from the University of Exeter, UK.

To close critical knowledge gaps we are seeking the support of our colleagues with collecting data on proportions, prevalence and types of marine turtle entanglement occurring globally. We hope that this information can be used to gather insight into the scale of this threat, focus future research needed for management and conservation for marine turtles faced by debris entanglement.

We are defining “marine turtle entanglement” as when a marine turtle has become entwined or trapped within any man made materials.

If you agree to participate in this study you are invited to complete this online questionnaire that will ask for your knowledge of the numerical scale and the severity of this issue when regarding stranded turtles. The survey can take 5-10 minutes and contains 20 key questions.

However, we encourage you to expand and provide us with any specific cases or photo images of such incidents; these would be greatly appreciated to help add more detail.

To increase the effectiveness and scope of our study we also actively encourage you to pass this survey onto your peers and colleagues that may have the knowledge to complete this survey.

Publication: The data from this survey will be used in the PhD thesis of ED and hopefully a manuscript on a global review on entanglement in marine turtles. Your responses and contact details will be strictly anonymous and not individually identifiable.

Thank you very much.

Informed Consent Approval

I understand that the aim of this research study is to collect data on proportions, prevalence and types of marine turtle entanglement. I consent to participate in this project and the details have been fully explained to me. I understand that my participation will involve completing the following online survey and I agree that the answers can be used in academic work and publications explained previously. I acknowledge that: - taking part in this study is voluntary and I am aware that I can stop taking part in it at any time without explanation or prejudice and to withdraw any unprocessed data I have provided. - any information I give will be kept strictly confidential and that no names will be used to identify me with this study without my approval. By clicking "Yes" in the check box below, I consent to completing this online questionnaire (Please tick to indicate consent).

- Yes
- No

1. **Name**
2. **Organisation:**
3. **Email:**

4. **Which ocean basin does your work primarily concern**?

- Atlantic
- Pacific
- Mediterranean
- Caribbean
- Indian

5. **In which country is your work based?**

6. **In which state/region/territory is your work based?**

7. **On average how many turtle strandings do you observe annually at this site (as stated above)?**

8. **For how many years have you being dealing with stranded turtles at this site?**

9. **Of these what is the species breakdown? I.e. what is the percentage for each species? Note they are listed alphabetically.**

Grid response: Species (Flatback, Green, Hawksbill, Kemp’s ridley, Leatherback, Loggerhead, Olive ridley) against percentage classification (0, 1-10, 11-20, 21-30, 31-40, 41-50, 51-60, 61-70, 71-80, 81-90, 91-100, N/A, Unsure)

10. **Approximately what percentage of all strandings are alive?**

Grid response: Species (Flatback, Green, Hawksbill, Kemp’s ridley, Leatherback, Loggerhead, Olive ridley) against percentage classification (0, 1-10, 11-20, 21-30, 31-40, 41-50, 51-60, 61-70, 71-80, 81-90, 91-100, N/A, Unsure)

11. Do you receive stranded sea turtles (or reports of) which are “entangled” (entwined or trapped) in man-made marine debris?

- Yes
- No
- Other:

12. If so what percentage of stranded sea turtles are “entangled” out of all strandings?

Grid response: Species (Flatback, Green, Hawksbill, Kemp’s ridley, Leatherback, Loggerhead, Olive ridley) against percentage classification (0, 1-10, 11-20, 21-30, 31-40, 41-50, 51-60, 61-70, 71-80, 81-90, 91-100, N/A, Unsure)

13. Approximately what percentage of "entangled" animals are still alive?

Grid response: Species (Flatback, Green, Hawksbill, Kemp’s ridley, Leatherback, Loggerhead, Olive ridley) against percentage classification (0, 1-10, 11-20, 21-30, 31-40, 41-50, 51-60, 61-70, 71-80, 81-90, 91-100, N/A, Unsure)

14. What kinds of materials have you experienced entangling stranded turtles? Please note, it is useful to differentiate whether fishing gear appeared to be lost/discarded or not.

Grid response: Entangling material (Lost/discarded fishing net, Lost/discarded fishing rope, Lost/discarded fishing line, Active fishing net, Active fishing rope, Active fishing line, Non fishing rope/twine, Woven sacks, Polythene sheets, Other) Other please explain/describe:

15. Which life stages are “entangled”? (Please select all that apply)

Grid response: Species (Flatback, Green, Hawksbill, Kemp’s ridley, Leatherback, Loggerhead, Olive ridley) against Life stage (Pelagic juveniles, Neritic juveniles, Adults, Pelagic & neritic juveniles, Pelagic juveniles & adults, Neritic juveniles & adults, All, N/A, Unsure)

16. Do you think the prevalence of entanglement has changed over the last 5 years?

- Increasing
- About the same
- Decreasing
- Other please explain/describe

17. Do you think the prevalence of entanglement has changed over the last 10 years?

- Increasing
- About the same

- Decreasing
- Other please explain/describe

18. Would you have images and specific cases that you would be prepared to share?

19. Are there any other peers/colleagues/organisations you can suggest to contact further the investigation?

20. Additional comment/information:

Box S2. Second Questionnaire

Turtle Entanglement - Sharing Results and Thoughts

Thank you so much for participating in our first Marine Turtle Entanglement survey. We received 106 responses from 50 countries and territories

In order to gain further insights into the challenges faced by this expert community and identify opportunities for more effective solutions, it would be great if you could have a look at our key findings and answer the following questions.

If you agree to participate in this study you are invited to complete a second online questionnaire that will ask for your expert knowledge on the issue of marine turtle entanglement. The survey can take 5-10 minutes and contains 10 key questions.

Publication: The data from this survey will be used in the PhD thesis of ED and hopefully a manuscript on a global review on entanglement in marine turtles. Your responses and contact details will be strictly anonymous and not individually identifiable.

Thank you very much.

Informed Consent Approval

I understand that the aim of this research study is to collect further information on the results from the previous Marine Turtle Entanglement survey on proportions, prevalence and types of marine turtle entanglement. I consent to participate in this project and the details have been fully explained to me. I understand that my participation will involve completing the following online survey and I agree that the answers can be used in academic work and publications explained previously. I acknowledge that: - taking part in this study is voluntary and I am aware that I can stop taking part in it at any time without explanation or prejudice and to withdraw any unprocessed data I have provided. - any information I give will be kept strictly confidential and that no names will be used to identify me with this study without my approval. By clicking "Yes" in the check box below, I consent to completing this online questionnaire (Please tick to indicate consent).

- Yes
- No

1. Name:

2. Organisation:

3. Email:

4. a) Is there anything missing form our results that you were expecting to see?

b) Was there anything in our results that was a surprise to you?

5. What do you think are the top three challenges to addressing entanglement issues in turtles?

6. What do you think are the three key research needs to better understand turtle entanglement?

7. What do you think would be the top three priority actions that would help reduce turtle entanglement?

8. How likely is entanglement in man-made debris to be causing population level effects in marine turtles?

- Definitely
- Very likely
- Probably
- Probably not
- Definitely not
- Don’t know

If so for what species and in which region (can provide multiple answers)?

9. How do you think the threat of entanglement in man-made debris compares to:

Grid response: Threat type (Plastic ingestion, Oil pollution, Fisheries bycatch, Direct exploitation, Climate change) against Threat level (Greater than entanglement, About the same as entanglement, Less than entanglement, Unsure)

10. Lastly, are there any questions you would like to ask of us?

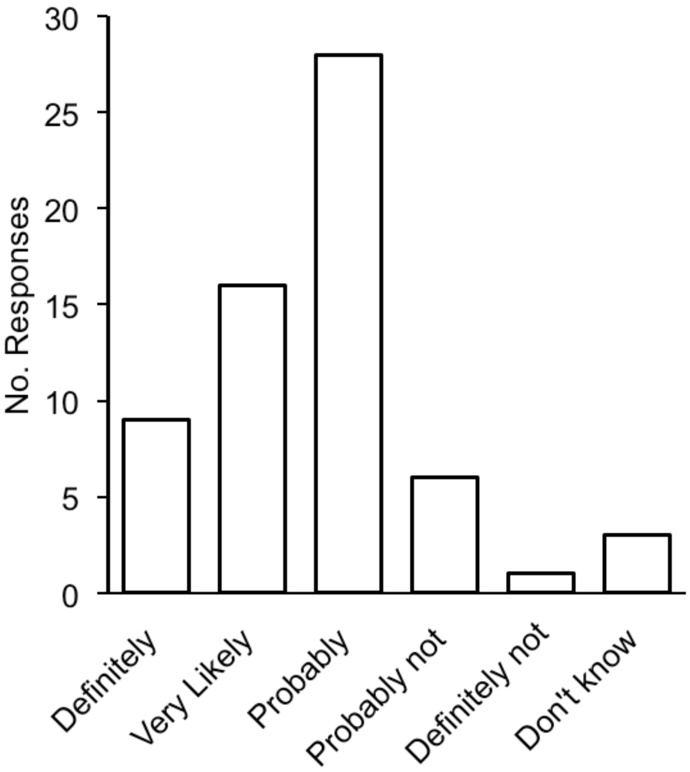

Figure S1. Likelihood of population level effects. Number of responses from experts when asked how likely entanglement in man-made debris to causing population level effects in marine turtles (n=63).

Chapter 4. Diet-related selectivity of macroplastic ingestion in marine turtles

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Abstract

Understanding the drivers of key interactions between marine vertebrates and plastic pollution is a considered research priority. Sea turtles are primarily visual predators, with the ability to discriminate according to colour and shape; allowing these factors to play a role in feeding choices. Ingested plastic classification methodologies currently lack records of these variables, however here, refined protocols allow us to test the hypothesis that plastic is selectively ingested when it resembles food items of green turtles (*Chelonia mydas)*. Turtles displayed strong and statistically significant diet-related selectivity towards certain types (sheet and thread), colours (black, clear and green) and shapes (linear items strongly preferred) of plastic when compared to the environmental baseline of plastic beach debris. There was a significant negative relationship between size of turtle (curved carapace length) and number/mass of plastic pieces ingested, which may be explained through naivety and ontogenetic shifts in diet. Additionally, the relationship between size (indicative of gape size of turtle) and mean length of ingested plastic was significant. Further species specific visual recordings would give greater insight into the selectivity of sea turtles in relation to ingested plastics based on a variety of physical properties. Thus advancing our knowledge as to the mechanisms of how impacts of marine plastics may manifest on vulnerable species.

Introduction

The abundance and spatial distribution of plastic pollution in the world’s oceans is ever increasing, recently becoming one of the most ubiquitous and long-lasting changes in natural systems (Barnes et al. 2009, Vegter et al. 2014, Jambeck et al. 2015). Extremely high densities of these novel pollutants are deposited along coastlines and in oceanic gyres (Watts et al. 2015). Plastic debris enters the marine environment via a variety of pathways; the major source being terrestrial runoff (accounting for an estimated 80%) but additional sources include fisheries and maritime activities (Andrady 2011).

The ingestion of plastic debris by marine vertebrates is now a global phenomenon. It is thought to occur in at least 43% of cetacean species, 36% of the seabird species, many species of fish and has been reported in all species of marine turtle (Campani et al. 2013, Schuyler, Hardesty, et al. 2014, Rees et al. 2016, Nelms et al. 2016). Plastics are the most commonly ingested of all anthropogenic debris; with a wide variety of items found inside necropsied sea turtles (Schuyler et al. 2012, Hoarau et al. 2014, Clukey et al. 2017, Pham et al. 2017, Vélez-Rubio et al. 2018). This has the potential to cause lethal effects from intestinal blockages and injuries but additionally adverse sub-lethal effects such as dietary dilution, malnutrition and impaired immunity (reviewed by Nelms et al. 2016). Although debris ingestion in these species is considered a global research priority, the specific drivers and the levels of mortality caused are still poorly understood (Hamann et al. 2010, Santos et al. 2015, Rees et al. 2016).

When attempting to understand reasons for plastic ingestion it is important to consider the feeding ecology of marine turtles (Schuyler et al. 2012, Fukuoka et al.

2016, Vélez-Rubio et al. 2018). Consumption of plastic maybe due to a failure of discrimination when mixed with normal dietary items. In juvenile green turtles in Brazil, plastic ingestion was thought to have occurred in conjunction with that of macroalagae due to debris entanglement with algal structures(Schuyler, Wilcox, et al. 2014, Di Beneditto & Awabdi 2014). On the other hand, individuals may be actively selecting items, for instance, leatherback turtles (*Dermochelys coriacea*) are known to ingest plastic bags resembling jellyfish (Mrosovsky et al. 2009). Furthermore a high occurrence of plastic bottle lids ingested by loggerhead turtles is thought to be because their round shape and presence floating near the surface resemble organisms normally preyed upon (Hoarau et al. 2014).

To promote an understanding of plastic ingestion in marine turtles, efforts have been expended towards documenting its prevalence. The EU Marine Strategy Framework Directive (2010) descriptor 10 included recommendations on future monitoring, suggesting loggerhead sea turtles would serve as a good indicator species to monitor ecological quality within European waters if data on ingestion could be collected from stranded or bycaught specimens (Galgani et al. 2014, Darmon et al. 2016). Building upon this, the Fulmar Protocol (the indicator species for the North Sea) (van Franeker et al. 2011) “toolkits” were created to unify methods for investigating plastic ingestion, allowing focus upon the differentiation between sources of ingested plastics (Campani et al. 2013, Camedda et al. 2014, Matiddi et al. 2017) i.e. the type of plastics ingested and their properties.

However, colour and especially shape are variables currently lacking in classification methodologies, receiving only negligible coverage within literature (Mascarenhas et al. 2004, Frick et al. 2009, Matiddi et al. 2017). Sea turtles are primarily visual feeders and an ability to discriminate between colour and shape hasbeen shown to play a role in feeding choices (Swimmer et al. 2005, Schuyler et al. 2012). Monitoring these aspects may offer insight into whether turtles are selectively ingesting some plastics. Data from beach plastic surveys have been used to set environmental baselines to investigate differences and selectivity with benthically feeding green (*Chelonia mydas*) and hawksbill (*Eretmochelys imbricata)* turtles, which show a strong preference for ingesting clear sheet or rope like plastics and avoiding harder coloured pieces (Schuyler et al. 2012).

Using data from stranded turtles we set out to test whether green turtles in the Eastern Mediterranean were selectively ingesting plastic that resembled their dietary items, typically seagrasses and algae (Bjorndal 1980).

Materials & Methods

Study Area

This study was conducted on the island of Cyprus, in the Eastern Mediterranean basin. The island hosts important nesting beaches and foraging grounds for the Mediterranean population of green turtles (*Chelonia mydas*)(Broderick et al. 2002). The coastline is regularly patrolled for nest monitoring and for stranded turtles, as well as having fisheries focused research and public awareness raising activities that led to the discovery, reporting and transportation of stranded or bycaught turtles to the author team for necropsy. The majority of samples are considered to have resulted from bycatch incidents in coastal small-scale fisheries, typically being drowned in bottom-set trammel nets (Snape et al. 2013).

Necropsy and gut content analysis

During 2014-2016, nineteen stranded or bycaught dead turtles, with curved carapace length (min CCL i.e. notch to notch) ranging between 25 and 86cm (36.9 ± 14.2 cm; mean ± SD; n=19) were recovered. The animals were subject to necropsy where biometric parameters were taken (Wyneken 2001).

During necropsy the entire gastrointestinal tract was removed and subdivided into 3 parts: oesophagus, stomach and intestine. These sections of the gastrointestinal (GI) tract were analysed separately, initial contents were weighed and then rinsed through a 1mm mesh sieve. After this, the remaining matter in the sieve was emptied into trays for sorting. Dietary items were separated, weighed and identified, meanwhile suspected plastic or other marine debris was removed, cleaned and dried (to obtain dry mass) mass and stored for later analysis. For selectivity analysis these whole gut samples were augmented with stomach content samples from nesting seasons 2011-2013 (n=15) to allow for a larger sample of ingested debris when focusing it’s the physical properties. These were not included in total measures of plastic ingestion in individual turtles due to lack of intestinal contents.

Novel Plastic Classification Methodology

The novel classification used in this study builds upon the Fulmar Protocol and MSFD (Marine Strategy Framework Directive) Marine Litter Report 2011 (Descriptor 10) “toolkits”. This involves categorising plastic debris into the following: Industrial plastic pellets or nurdles (IND) and user plastics (USE) which can be split into several sub-categories; sheetlike plastics (SHE) e.g. plastic bags, threadlike plastic (THR) e.g. remains of rope, foamed plastics (FOAM) e.g. polystyrene, fragments (FRAG) e.g. hard plastic items and other (POTH) e.g. rubber, elastics, items that are ‘plastic-like’ that do not clearly fit into another category. With dry weight (mg) taken of every individual piece isolated (van Franeker et al. 2011). Additional recordings of colour, shape and three dimensional measurements of each individual piece of plastic were also taken. Colour was recorded within 11 categories; Clear, White, Pink/Purple, Red, Orange, Yellow, Green, Blue, Brown, Black, Grey. To gain an environmental baseline, 17 beaches distributed around the coastline were sampled between July-August 2016 for deposited plastic marine debris (see Supporting information). Beach survey is regarded as the simplest and most cost- effective method to provide a reasonable proxy for marine debris environmental availability (Ryan et al. 2009).

Statistical Analysis

The frequency of occurrence and relative abundance for each plastic type and colour category was calculated as per Schuyler et al*.* (2012). We calculated Manly's selectivity ratio for debris type and colour. In the past this method has been used widely to estimate for habitat or diet selection but more recently has been used to explore the selectivity of plastic debris because the index takes into account the availability of each debris type and colour in the environment (Schuyler et al. 2012). If the value calculated is >1 this indicates a positive selectivity for that type/colour category, suggesting that turtles target that type of plastic compared to what is available in the environment. However a value <1 indicates a negative selectivity to that category, suggesting avoidance of that debris type in the environment. Width: Length ratios were calculated (W/L) for all 1364 pieces ingested by green turtles and 1167 pieces of beach plastic debris. A ratio close to 1 indicated a square or round 2D piece of debris with ratios <1 leading to rectangular and progressively more linear shapes with decreasing ratio.

Results

Abundance of ingested plastic

All green turtles, where whole GI tracts were available (n=19), had ingested plastics with individuals having ingested an average of 61.8 items (± 15.8 mean ± SE); ranging from 3-183 pieces (weighing an average of 1.76 ± 0.53g; ranging from 0.04-7.93g) (Figure 1a). The majority of this plastic debris was found in the intestine section (100% occurrence) compared to the oesophagus (22%) or the stomach (33%) sections. For individuals for only stomach content samples was available (n=15) 27% contained ingested plastic.

There was a significant relationship between curved carapace length and the number (r^S= -0.658, n=19, p=0.002) (Figure 1b) and mass of ingested plastic (g) (r^S= -0.592, n=19, p=0.008). In addition there was a significant relationship between size (indicative of gap size of turtle) and mean length of ingested plastic (r^S=0.553, n=19, p=0.014) but not mean area of ingested plastic (r^S=0.219, n=19, p=0.369).

Diet-related selectivity

In relation to the ingested plastic, Manly's selectivity ratio highlighted a selectivity compared to environmental availability (Figure S1). Calculated ratios showed green turtles exhibited a very strong selectivity towards both SHE and THR (wi=7*.033*, wi=6.968) plastic debris but appearing to avoid ingestion of FOAM, FRAG, POTH (e.g. rubber) and IND types (Figure 2a). When considering the ingestion of certain colour categories of plastic the green turtles showed strong selectivity for *black*, *clear* and *green* debris (wi=2.457, wi=1.629, wi=1.234) and also slight selectivity for *pink/purple*, *brown* and *yellow* debris while showing avoidance of *white*, *red*, *grey*, *orange* and *blue* plastics (Figure 2b). In terms of debris shape plastic with a small width: length ratio (long rectangular) were ingested at the highest frequency with turtles showing strong selectivity for lowest w/l ratios (wi=3.823) and weak selectivity/partial avoidance to higher w/l ratio values (more square or round) (Figure 2c).

Discussion

Key results

The current work suggests that green turtles (particularly juveniles) foraging in coastal waters of Cyprus regularly encounter and ingest plastic, so much so that the vast majority of animals contained some plastic in their GI tract, that demonstrates diet-related selection, at the time of their death. Given the conservation status of this endangered species in the Mediterranean region (Wallace et al. 2011), that consumed marine plastics are considered to have negative fitness impacts, and the high prevalence of plastics in the Mediterranean region, this is an important finding.

Diet-related selectivity

Selective ingestion of plastic is plausible for green turtles as they have been shown to be capable of choosing particular species of seagrass over others or tending “grazing plots” therefore being selective in their natural feeding ecology (Bjorndal 1980). Strong selectivity was exhibited towards plastics that potentially resemble their main dietary item, sea grass. Firstly, plastics types that were more preferably ingested were softer, more pliable plastics that tended to have a smaller width: length ratio therefore resembling sea grass by shape and texture. Additionally the colours selected for were *black*, *clear* and *green*, these colours more closely resemble sea grass. Similarly, green turtles from Australia showed a strong preference for ingesting clear sheet or rope like plastics, avoiding harder coloured pieces (Schuyler et al. 2012). This indicates that turtles may not just be selecting plastics that look like gelatinous prey, which has been commonly stated in the literature as the “jellyfish hypothesis”, but other prey items. This explanation has been being previously shown to be inconsistent with the diversity of ingested plastic and their similarity to the main dietary items found in green turtles (Derraik 2002, Schuyler, Wilcox, et al. 2014, Santos et al. 2015, 2016, Fukuoka et al. 2016, Vélez-Rubio et al. 2018).

Secondly, turtle visual biology and perception of colour could also greatly influence the ingestion of particular types or colours of debris (Fukuoka et al. 2016). Thayer's law of countershading colouration in nature has been used to infer the likelihood of turtles detecting plastic fragments in the water column (Santos et al. 2016). Santos et al. (2016) suggest that marine animals that perceive floating plastic from below should preferentially ingest dark plastic fragments, whereas animals that perceive floating plastic from above should select for paler plastic. Our results for eastern Mediterranean green turtles are consistent with their study on Brazilian green turtles, with floating darker debris (black, green) ingested over proportions found in environmental available. However our study also showed preferential ingestion of clear plastics. Perhaps it is more plausible that biofouled clear plastics that have sunk to the seafloor could be perceived from above if a turtle is foraging benthically or mid-water column (Santos et al. 2016).

Size and ingested plastic

Size class or life history stage appears to be an important factor in determining the probability or variability of plastic ingestion. This may be a result of the feeding ecology and ontogenetic shifts in diet known in this species. During the early oceanic juvenile stage turtles develop an opportunistic feeding strategy, aggregating at frontal zones (Bolten 2003), after which they recruit to neritic habitats and develop a more herbivorous diet principally based on seagrass and algae (Mortimer 1981). However, some retain an omnivorous, less specialised diet for longer, which could explain variable ingestion of plastic debris within this life stage due to differences in the ontogenetic timing of diet specialisation (Bjorndal 1997, Seminoff et al. 2009, Shimada et al. 2014, Vélez-Rubio et al. 2018).

This relationship of has been also highlighted with in other studies. In both green and hawksbill turtles from the Queensland coast, Australia, the probability of debris ingestion was inversely correlated with size, with smaller pelagic turtles significantly more likely to ingest debris than larger benthic feeding turtles (Schuyler et al. 2012). Indeed it has previously been argued that plastic ingestion by juvenile marine turtles is an underestimated problem, with surprisingly small amounts of debris sufficient to fatally block the digestive tract (Santos et al. 2015). This might have other longer term consequences that could include reduced growth rates, fecundity, reproductive success, and late sexual maturation which could have demographic ramifications (Hoarau et al. 2014, Vegter et al. 2014, Nelms et al. 2016). Future studies should aim to assess the impact on these particularly susceptible life stage.

Importance of a Unified Classification System

To date, there have been relatively few studies within the Mediterranean on plastic ingestion by green turtles compared to current literature on the status of this threat in the loggerhead turtle population; where ingestion rates vary between 5-75% (Tomas et al. 2002, Campani et al. 2013, Camedda et al. 2014, Matiddi et al. 2017). When comparing ingestion rates for the green turtles to those seen globally these are equivalent to some of the highest observed (in Brazil and others parts of South America) (Guebert-Bartholo et al. 2011, González Carman et al. 2014, Santos et al. 2015, Fukuoka et al. 2016, Vélez-Rubio et al. 2018). Currently the loggerhead turtle isthe only indicator species for plastic ingestion in the Mediterranean for the Marine

Strategy Framework Directive (GES Technical Subgroup on Marine Litter). However our results highlight the importance of not focusing on a single indicator species to obtain a true indication of the impacts of this pollutant (Galgani et al. 2014, Matiddi et al. 2017)This is confounded by the fact that current methodological differences between studies limit comparison of the debris ingestion in sea turtles.

There is no unified, globally used, classification system of ingested plastics in marine turtles. Many recent studies focus upon the debris occurrence (%), however, factors potentially determining differences are overlooked, such as the characteristics of ingested plastic (Casale et al. 2016). The unification of plastic classification and the use of a singular categorisation method with in the field would greatly aid intra- and inter- species comparisons and additionally in comparisons with other taxa known to be effected by marine debris (Pham et al. 2017). For example, the investigation of plastic ingestion in seabirds has benefited from the adoption of the Fulmar protocol globally, with classification systems proving a cost effective biomonitor both in Europe and the North Pacific (Avery-Gomm et al. 2012). Simply removing stomach contents to sample for macroplastic ingestion as initially suggested by Bjorndal et al. (1994) load is not ideal as much of the retention of plastics occurs within the intestines, with the anterior portion of the rectum being shown to have the highest number of obstructions in this species (Casale et al. 2016).

In conclusion, green turtles displayed strong diet-related selectivity towards certain types, colours and shapes of plastic when compared to the environmentally available baselines, preferentially ingesting certain items even when they are less readily available in the environment. Colour and shape are factors that feed into the turtle’s foraging decision making. This study adds further support to the “active selectivity” hypothesis of plastic ingestion over the “accidental/ opportunistic” hypothesis that has also been proposed within the literature (Schuyler et al. 2012, Di Beneditto & Awabdi 2014). To understand the mechanisms of the “active selectivity” hypothesis, it is important to link this with known developmental biology and feeding ecology. Further species specific visual recordings would give greater insight into the selectivity of sea turtles in relation to ingested plastics based on a variety of physical properties. Thus would lead to advances in this particular field and guide future research enabling the implementation of targeted conservation management strategies (Schuyler, Wilcox, et al. 2014).

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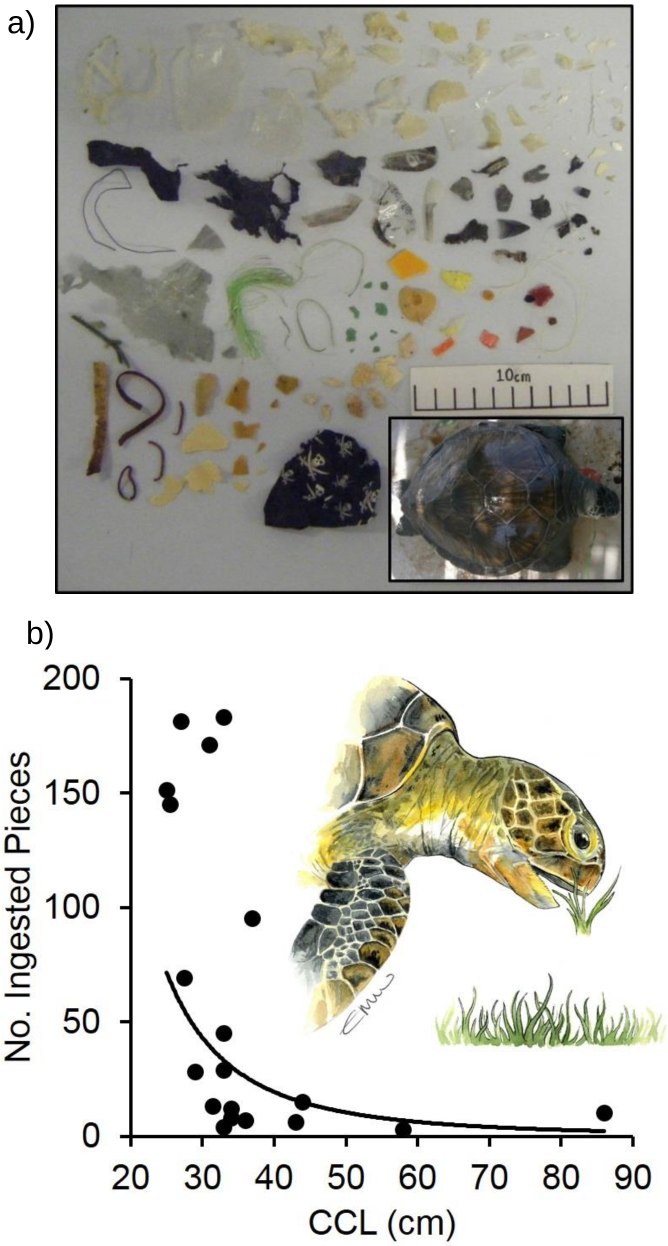
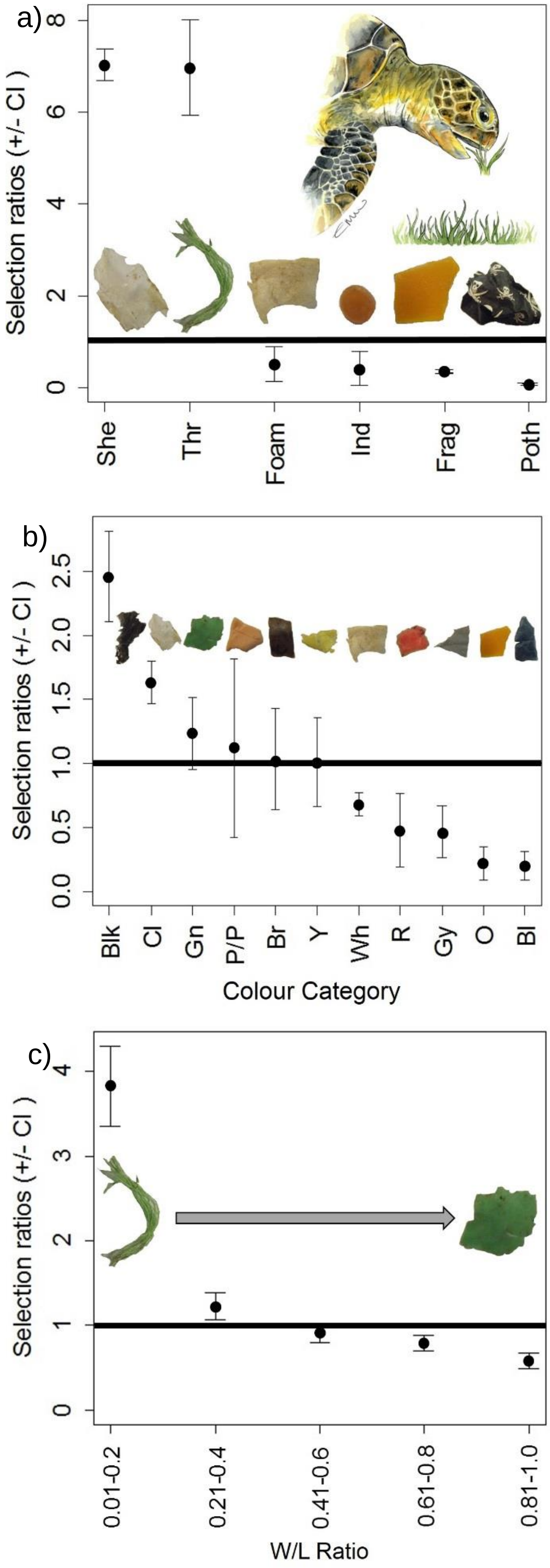


Figure 1. Macroplastic ingestion in green turtles (*Chelonia mydas*) from the Eastern Mediterranean. a) Ingested plastic removed from the intestine of a juvenile (CCL=33cm) showing the high quantities and diversity of plastic debris ingested. b) Curved carapace length (cm) vs. the number of ingested pieces of plastic (n=19).


a)
Selection ratios (+/- CI)
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Colour Category
c)
Selection ratios (+/- CI)
0
1
2
3
4
0.01-0.2
0.21-0.4
0.41-0.6
0.61-0.8
0.81-1.0
W/L Ratio

Figure 2. Marine turtle diet-related selectivity in macroplastic ingestion in the green turtles (*Chelonia mydas*) (n=34). Manly's Selectivity Ratios. A value >1 this indicates a positive selectivity for that type/colour category than availability in the environment. Error bars indicate 95% confidence intervals. a) type of plastic debris SHE=sheetlike plastics, THR=threadlike plastics, FOAM= foamed plastics, FRAG= hard plastics, POTH= other 'plastic like' items, IND= industrial nurdles b) colour of plastic debris. Cl=Clear, Blk=Black, Y=Yellow, Wh=White, Gn=Green, Bl=Blue, Br=Brown, Gy=Grey, O=Orange, P/P=Pink/Purple, R=Red. c) width/length ratio. If the ratio number produced was <0.2 this represented a rectangular shape whereas a ratio close to 1 indicated a more square or circular piece of debris.

Chapter 4: Supplementary Information

Beach surveys

Study Area

Sampling was carried out at 17 beaches along the north and east coast of Cyprus, Eastern Mediterranean between July and August 2016. Surveys were organised to coincide with the main period of turtle nesting/hatching activity. Beaches were selected, based upon their spatial distribution and high turtle nesting densities.

Sediment Sampling

Within each beach, data were collected from 10 pairs of sampling sites along two lines parallel to the shore: the ‘strandline’ and “transect of typical turtle nesting area”. Strandline (SL) was defined as the highest line of debris left from the retreating tide. This meandering line where debris accumulates is periodically generated by tide and exposed air movements (Heo et al. 2013); the transect through turtle nesting area was approximately the median distance between strandline and the landward limit of the beach within which turtles nested, approximated by a) marked nests, b) body pits left from nesting attempts.

The 10 sampling sites were spaced equidistantly, with sample 1 and 10 lying 5% of the beach length from each end to avoid rocky edges of the beach.

All samples were collected using a cylindrical metal corer of 20cm diameter and 60cm height. All sand was gathered for 0-2cm depth at sampling locations on the strandline and the nesting area. At locations in the nesting area a volume of 250cm^3 was taken from incremental depths (2.1-10.0cm, 10.1-20.0, 20.1-30.0, 30.1-40.0, 40.1-50.0, 50.1-60.0cm) unless water or rock was struck first. SL samples were taken from the surface down to a matching 2cm depth, to allow for comparisons with recent similar studies. Each subsample was air dried in metal trays before being sieved.

Plastic Separation & Categorisation

All samples of sediment were air dried. Dry weight of sediment subsamples was measured to an accuracy of 0.01g, before being passed through a sieve cascade of a 5mm and 1mm mesh respectively. This allowed capture of plastics within the micro category defined as < 5mm. Anthropogenic waste of 5-200mm sizing was also gathered from the top mesh (5mm) defined as macroplastic (X>5mm), the size class used as the environmental baseline to this study.

From each sieve layer plastic debris were removed by eye to be analysed and categorised by the classification method stated as set out by the Fulmar Protocol and MSFD (Marine Strategy Framework Directive) Marine Litter Report 2011 (Descriptor 10) “toolkits” including type and colour of plastics (Galgani et al. 2014). To gain a baseline for shape and size of plastics this dataset was augmented with further beach surveys during August 2017 (n=1167pieces >0.5cm).

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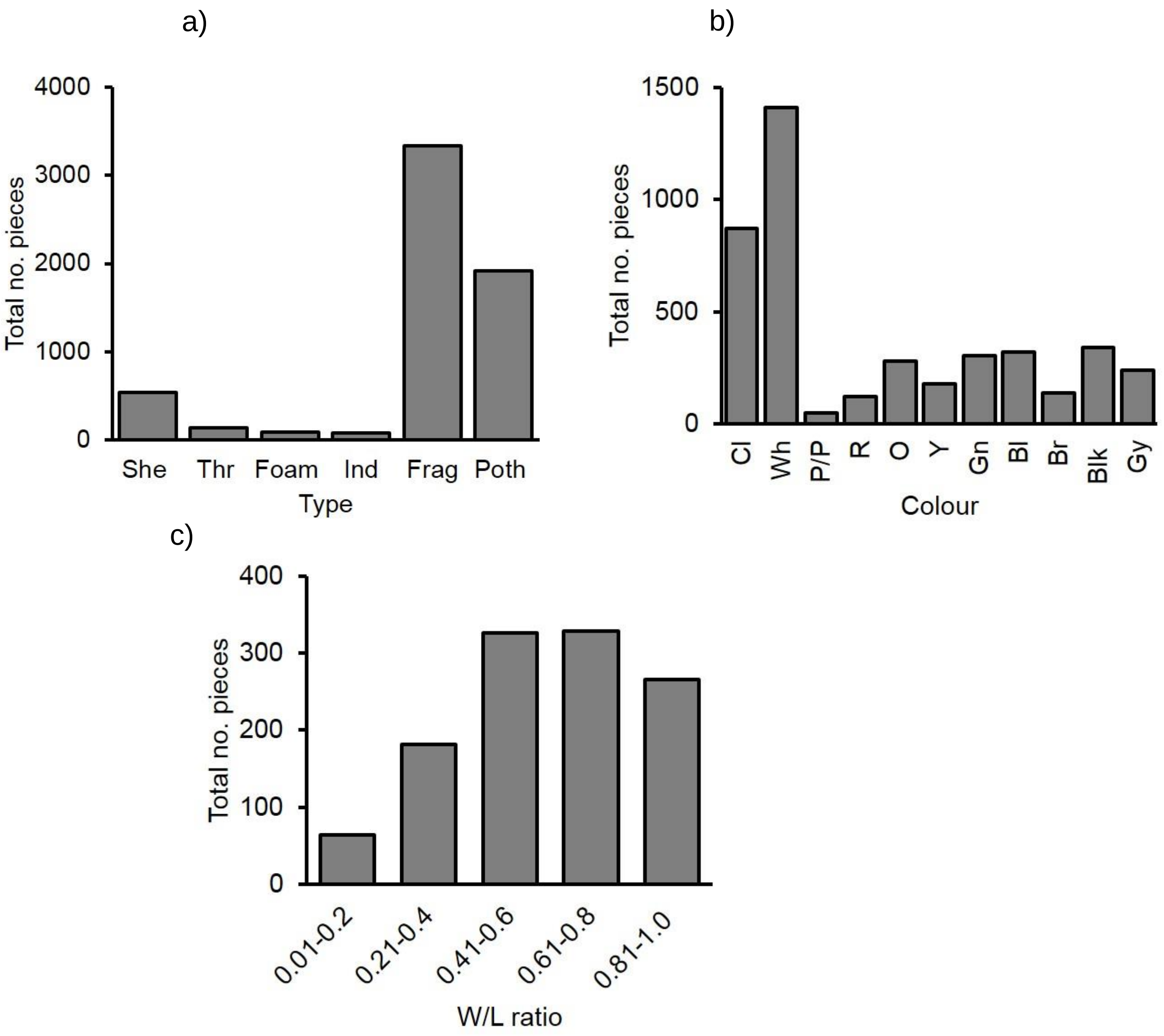

Figure S1. Type, colour and size of plastic debris from beach surveys (n=1167) a) type of plastic debris SHE=sheetlike plastics, THR=threadlike plastics, FOAM= foamed plastics, FRAG= hard plastics, POTH= other 'plastic like' items, IND= industrial nurdles b) colour of plastic debris. Cl=Clear, Blk=Black, Y=Yellow, Wh=White, Gn=Green, Bl=Blue, Br=Brown, Gy=Grey, O=Orange, P/P=Pink/Purple, R=Red. c) width/length ratio. If the ratio number produced was <0.2 this represented a rectangular shape whereas a ratio close to 1 indicated a more square or circular piece of debris.

Chapter 5. Microplastic ingestion ubiquitous in marine turtles

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Abstract

Despite concerns regarding the environmental impacts of microplastics, knowledge of the incidence and levels of synthetic particles in large marine vertebrates is lacking. Here we utilize an optimised enzymatic digestion methodology, previously developed for zooplankton, to explore whether synthetic particles could be isolated from marine turtle ingesta. We report the presence of synthetic particles in every turtle subjected to investigation (n=102) which included individuals from all seven species of marine turtle, sampled from three ocean basins (Atlantic (ATL): n=30, 4 species; Mediterranean (MED): n=56, 2 species; Pacific (PAC): n=16, 5 species). Most particles (n=811) were fibres (ATL: 77.1%: MED: 85.3% PAC: 64.8%) with blue and black being the dominant colours. In lesser quantities were fragments (ATL: 22.9%: MED: 14.7% PAC: 20.2%) and microbeads (4.8%; PAC only; to our knowledge the first isolation of microbeads from marine megavertebrates). Fourier transform infrared spectroscopy (FT-IR) of a sub-sample of particles (n=169) showed a range of synthetic materials such as elastomers (MED: 61.2%; PAC: 3.4%), thermoplastics (ATL: 36.8%: MED: 20.7% PAC: 27.7%) and synthetic regenerated cellulosic fibres (SRCF; ATL: 63.2%: MED: 5.8 % PAC: 68.9%). Synthetic particles being isolated from species occupying different trophic levels suggests the possibility of multiple ingestion pathways. These include exposure from polluted seawater and sediments and/or additional trophic transfer from contaminated prey/forage items. We assess the likelihood that microplastic ingestion presents a significant conservation problem at current levels compared to other anthropogenic threats.

Introduction

Plastic debris is ubiquitous in the marine environment (Rochman et al. 2015). It is estimated that 4.8 to 12.7 million tonnes of plastic waste could be entering the marine environment annually, contributing to an estimated five trillion pieces of plastic in the surface waters of the global seas (Eriksen et al. 2014, Jambeck et al. 2015). Recently there has been a growing concern regarding “microplastics”, which are defined as plastic particles <5mm. Due to their high abundance and bioavailability, microplastics have been considered as a pollutant in their own right (Andrady, 2011; Cole, 2014).

Primary microplastics are most commonly associated with exfoliators in cosmetic products, or pre-production nurdles but can also result from “microbead” use in biomedical applications, air-blasting technology, automotive tyre wear, or fibres from the breakdown of clothing (Derraik 2002, Cole et al. 2011, Napper et al. 2015, Napper & Thompson 2016, Nelms et al. 2017). Secondary microplastics are derived from the disintegration of larger plastic items (“macroplastics”) within marine systems through wave action, UV radiation exposure and physical abrasion as the items are moved by wave action, or washed over shorelines. The cumulative effects of these physical, biological and chemical processes reduce the structural integrity of the plastic and result in fragmentation of the items into smaller, eventually microscopic particles (Browne et al. 2007).

Ingestion of microplastics is now being reported in a number of marine invertebrate species (Wright *et al.* 2013; Cole *et al.* 2014; Setälä *et al.* 2014; Watts *et al.* 2014; Long *et al.* 2017; Dawson *et al.* 2018; Foley *et al.* 2018). The possible physiological and ecological effects of ingestion for these species is beginning to be understood;

for example microfibre ingestion in crabs can affect food consumption and energy balance and ingestion of microscopic unplasticised polyvinylchloride (UPVC) reduces growth and energy reserves in marine worms (Wright et al. 2013, Watts et al. 2015). Descriptive reports are also starting to appear for vertebrates such as fish (Lusher et al. 2013, Rochman et al. 2015, Collard et al. 2015, Stolte et al. 2015, Güven et al. 2017, Foley et al. 2018) and marine mammals (Fossi et al. 2012, 2016, Lusher et al. 2018, Nelms et al. 2018).

Knowledge relating to the incidence of microplastic (<5mm) ingestion in marine turtles still remains very limited, despite records of all seven species of marine turtles ingesting macroplastics (>5mm) (Boyle & Limpus 2008, Schuyler et al. 2014, Hoarau et al. 2014, Nelms et al. 2016, Lynch 2018, Yaghmour et al. 2018) and the creation of global risk maps aiding in the identification of interaction hotspots (Schuyler et al. 2015). The only exception is the isolation of seven microplastic particles (<5mm) from the gut contents of two green (*Chelonia mydas*) turtles from the Great Barrier Reef (Caron et al. 2018) and recent accounts relating to stranded post-hatchlings from the Atlantic (White et al. 2018).

Rising concerns regarding global impacts of microplastic pollution on marine wildlife mandates a reliable and comparable detection protocol (Nelms et al. 2016). Here, alongside investigation of macroplastic ingestion (>5mm), we develop a methodology to explore whether synthetic particles (<5mm) could be isolated from marine turtle ingesta. We sought to: (1) identify the extent of microplastic ingestion in all species of marine turtles; and (2) explore the polymer type of any ingested particles.

Materials and Methods

Study Sites

The study was conducted in three ocean basins using both stranded and bycaught animals (n=102; all 7 marine turtle species. In the Mediterranean basin (MED) samples were collected from Northern Cyprus where stranded and bycaught green (*Chelonia mydas*) and loggerhead (*Caretta caretta*) turtles are common. In the Atlantic basin (ATL) samples were collected from North Carolina, USA which experiences strandings of green, loggerhead, Kemp's ridley (*Lepidochelys kempii*) and leatherback (*Dermochelys coriacea*) turtles. Finally the Pacific basin (PAC) with samples provided from Queensland, Australia which included stranded and bycaught post-hatchling green, loggerhead, flatback (*Natator depressus*), hawksbill (*Eretmochelys imbricata*) and olive ridley turtles (*Lepidochelys olivacea*) (Summarised in Table S1.; Figure 1.).

Necropsy and gut content analysis

Animals were subject to necropsy and biometric parameters were taken (minimum curved carapace length (CCL) (Bolten 1999). The entire gastrointestinal tract was removed and initial contents were weighed and then rinsed through a 1 mm mesh sieve. The remaining matter in the sieve was emptied into trays for sorting with macroplastic removed and stored for later analysis. A 100ml sample (approximately 5% of the total) of gut content residue and associated supernatant was collected from material that had passed through the 1 mm mesh sieve. This was later oven dried at 60˚C for 24 hours to enhance the efficacy of homogenization in later steps of the process. Gut content residue samples were exposed to an optimised enzymatic digestion protocol that had been developed for use on zooplankton material by Cole et al., (2014). Digestion filters were then analysed under a digital stereo microscope (Leica M165C) and classified by type, colour and size. A sub sample (n=169) of these identified particles were analysed using Fourier transform infrared spectroscopy (FT-IR) (Figure S2.). Extensive measures were taken to minimise possible sample contamination (For full details see Supplemental methods).

Results

Synthetic particle ingestion

Synthetic particles (<1mm) were identified in every individual (n=102) of all seven species over the three ocean basins, with 811 particles isolated in total. This 100% incidence contrasts with highly variable occurrence rates of macroplastic (>5mm) ingestion in some species in the study areas (range: 0-100%) (Figure 1.). Although sample sizes were small for some site-specific species groups, there was a marked variability of incidence in synthetic particle ingestion among sites, with levels appearing higher in turtles from the Mediterranean (Figure 2.).

Particle description

The type of particle varied among sites. The majority of these were classified as fibres at all three sites (ATL: 77.1%: MED: 85.3% PAC: 64.8%) and in lesser quantities were fragments (ATL: 22.9%: MED: 14.7% PAC: 20.2%) and microbeads (4.8%; PAC only) (Figure 3.). Fibres spanned several of the eleven colour categories (ATL: 4/11; MED: 10/11; PAC: 6/11) but the large majority of fibres were blue or black in all sites (Blue: ATL: 36.3%; MED: 34.4%; PAC: 44.9%; Black: ATL: 43.7%; MED: 31.3%; PAC: 39.1) followed by red and clear fibres (Red: ATL: 17.5%; MED: 18.2%; PAC: 8.6%; Clear: ATL: 2.5%; MED: 9.9%; PAC: 2.9%) (Figure 3.)

Polymer Identification

A sub-sample of 20% (n=169) of the isolated particles were tested using FT-IR to determine their polymer composition (Table S2.). This analysis revealed the majority were synthetic materials (n=160) (ATL: 100%; MED: 92.6%; PAC: 100%) with only a minority being naturally occurring materials such as natural rubber and plant protein (n=9) (MED: 7.4%). In addition, not all synthetic materials comprised plastic polymers. Our spectral matches identified elastomers (MED: 61.2%; PAC: 3.4%) such as Ethylene Propylene Diene Monomer (EPDM Rubber), Hydronated Nitrile Butadiene Rubber (HNBR) and Neoprene. We also identified woven synthetics (MED: 4.9%) such as polyaramid Kevlar® and synthetic regenerated cellulosic fibres (SRCF) e.g. rayon, viscose (ATL: 63.2%; MED: 5.8 %; PAC: 68.9%). Of the confirmed true microplastics (ATL: 36.8%; MED: 20.7%; PAC: 27.7%) we identified the spectral characteristics of Polyethylene, Ethylene Propylene, Polyester, with isolated microbeads being identified as Polyacrylamide.

Discussion

Synthetic particle ingestion in marine turtles

Here we have shown that synthetic particles including microplastics (<5mm) were present in every turtle, across all species and ocean basins sampled, even though not all individuals had ingested macroplastics. Sample sizes and methodology preclude in-depth analysis here but ingestion may be generally higher in the Mediterranean basin than the wider Atlantic or Pacific. Global models predict some of the world’s highest concentrations of marine plastics in this basin (Cózar et al. 2014, Eriksen et al. 2014, Suaria et al. 2016, Duncan et al. 2018). Further, more exhaustive sampling is required to fully appraise interspecific and geographic differences.

Most particles isolated in our analysis were fibrous in nature. Indeed fibres are now a prolific pollutant and are some of the most commonly observed in the natural environment; with numerous potential sources (Gago et al. 2018). In terms of colour,

our results mirror studies on plankton ingestion, environmental seawater and sediments, with the majority of fibrous microplastics being predominately black, blue or red (Güven et al. 2017, Steer et al. 2017, Gago et al. 2018). Sources of synthetic fibres include microfibre shedding from the mechanical and chemical stresses undergone by synthetic fabrics (Napper & Thompson 2016, De Falco et al. 2018), automotive tyre wear (Wagner et al. 2018) and degradation of cigarette filters and fragmentation of maritime equipment such as ropes and fishing nets (Napper & Thompson 2016; De Falco *et al.* 2018). Synthetic fibre ingestion has been documented in filter feeding marine invertebrates such as mussels, clams and zooplankton and are thought to be in some cases mistaken for natural prey items (Mathalon & Hill 2014, Davidson & Dudas 2016). However within marine turtles, due to the size of particles, ingestion is more likely to be through indirect mechanisms (ingestion pathways discussed further below) (Nelms et al. 2016).

Fragments were found as a minority in all three basins and microbeads were only identified in our samples from the Pacific Ocean. To our knowledge, this is the first isolation of microbeads from marine megavertebrates, being only identified in fish and planktonic gut content previously (Setälä et al. 2015, Tanaka & Takada 2016, Lusher et al. 2017, Steer et al. 2017, Peters et al. 2017). This could potentially be due to the foraging ecology of turtles sampled from the Pacific. Post-hatchlings are known to be epipelagic surface dwelling unlike their neritic coastal counterparts (Bolten 2003, Ryan et al. 2016, Clukey et al. 2017) leading to a spatial overlap with surface floating microplastics.

Microplastic polymer identification

The polymer make-up of marine plastic debris may aid in identifying possible sources, degradation, fate and reasons for ingestion (Jung et al. 2018, Nelms et al. 2018). The polymers identified through FT-IR analysis reflect the recently reported polymer diversity globally described for microplastics (Gago et al. 2018). Polyethylene (PE) and polypropylene (PP) are some of the most abundant polymers found as pollutants worldwide (White et al. 2018, Gago et al. 2018). Furthermore Suaria et al. (2016) identified sixteen classes of synthetic material from the surface waters of the central-western Mediterranean Sea. Within these classes, low-density polymers such as polyethylene and polypropylene were again abundant, followed less frequently by polymers such as polyethylene terephthalate, polystyrene and polyamides which were also identified in the marine turtle gut content of this study. However, in our study, a large proportion of synthetic samples in the Mediterranean, belonged to the class of elastomers (e.g. EPDM Rubber, HNBR Rubber, Nitrile-Butadiene Rubber). A major contributor to the presence of elastomers in the marine environment being tyre wear particles (TWP), with the majority of emission coming from road side run off (Wagner et al. 2018). Polyacrylamide microbeads described in our Pacific samples have been used in the past in drug delivery (El-Samaligy & Rohdewald 1982) and more recently for a number of biomedical applications such as encapsulation (Labriola et al. 2017). Alternatively these could originate from exfoliating agents in cosmetic products (Napper et al. 2015).

There are numerous challenges in studying microplastics in the environment including the analytical chemistry to identify particles (Comnea-Stancu et al. 2017, Silva et al. 2018)**.** Visual examination is the most common method used to identify microplastics. Although efficient, *in-situ* and low cost, there are several limitations, such as the inherent difficulty in distinguishing microplastics from other small particles, for example natural or synthetic materials. Many potential microplastic fibres from the FT-IR sub-sample in this study were identified with high spectral matches as cellulose based particles, despite their appearance under visual examination as microplastics. Indeed this has begun to be reported elsewhere within the literature (Remy et al. 2015, Cai et al. 2017, Courtene-Jones et al. 2017). For example blue cotton-indigo fibres from samples of waste water treatments plants can show close visual similarity to polyacrylic fibres (Dyachenko et al. 2017, Silva et al. 2018).

However, from further inspection of other digital photographs, individual spectra and high match qualities (over 80-90%) we propose that these are synthetic regenerated cellulosic fibres (SRCF) such as viscose or rayon. Although originally derived from natural sources they undergo several chemical processes in regeneration to become reconstructed (Comnea-Stancu et al. 2017, Gago et al. 2018). There are distinct differences between native and regenerated cellulose regarding their crystalline structure. These differences could affect their persistence in the marine environments, and hence their presence in marine turtle guts. Such SRCFs could represent a major fraction of fibres in the environment (Comnea-Stancu et al. 2017). Future research should aim to build protocols to accurately interpret outputs, to be able to distinguish between SRCFs and other natural materials as it is clear that visual inspection alone is insufficient.

Ingestion pathways

There are multiple potential ingestion pathways. Firstly, the presence of synthetic particles in marine turtles could be due to environmental exposure to areas of contaminated sea water or sediments. Numerous studies have now identified microplastics in seawater worldwide creating potential exposure during foraging, nesting and migration (van Sebille et al. 2015, Critchell et al. 2015, Gago et al. 2018). Microplastics have also been shown to move from source to sediments (Gago et al. 2018), with low-density plastics eventually reaching the seafloor though density-modification, as a result of biofouling or integration into zooplankton faecal matter (Andrady 2011, Cózar et al. 2014, Van Cauwenberghe et al. 2015, Alomar et al. 2016, Cole et al. 2016, Coppock et al. 2017). Many marine turtles are known to feed benthically, for example, benthic feeding loggerhead turtles actively rework sediments which are ingested along with their prey (Preen 1996, Casale et al. 2008, Lazar et al. 2011).

Another pathway of exposure could be from particles in or on primary producers and sessile filter feeders, when the feeding ecology of hawksbill and green turtles is considered (Bjorndal 1980, Obura et al. 2010, Bell 2013). For example microplastics can adhere to the surface of seaweeds electrostatically binding to cellulose or retention facilitated by a mucus layer on the surface (Gutow et al. 2016) and sponges are known to ingest microplastics (Baird 2016), creating a pathway of ingestion alongside dietary items.

Finally, synthetic particle presence in omnivorous life stages or species, especially loggerhead or ridley turtles, could originate through a pathway of trophic transfer from contaminated prey such as filter feeding invertebrates. Laboratory studies have shown trophic transfer of microplastics between invertebrates and within planktonic food webs (Farrell & Nelson 2013, Setälä et al. 2014, Dawson et al. 2018, Macali et al. 2018). In addition, a recent study by Nelms *et al.* (2018) on grey seals (*Halichoerus grypus*) and wild-caught Atlantic mackerel (*Scomber scombrus*) suggested that trophic transfer represents an indirect but potentially major pathway for any species whose feeding ecology involves the consumption of whole prey.

Potential impacts

We only tested a subsample of the gut content residue in our study and these represent a minimum count of the number of the gut burden. The total number of synthetic particles within the whole gut is likely to be the order of 20 times higher. This suggests that the total levels of ingestion per individual (whole gut) may be higher in marine turtles than large marine mammals. In a recent study focusing on cetaceans (n=21) stranded and bycaught individuals were found to contain plastic particles ranging from 1-88 in whole digestive tract samples. These were composed of the majority fibres (83.6%) and the remaining were fragments (16.4%) (Lusher et al 2018).

It remains unknown if and how these synthetic particles will impact turtles. Their size means they will pass through the gut lumen with relative ease (especially for larger specimens) and therefore their presence does not lead to blockage or obstruction which is frequently reported in association with macroplastic ingestion (Ryan et al. 2016). Importantly future work should focus on whether microplastics may be affecting aquatic organisms more subtly, e.g., exposure to associated contaminants (heavy metals, POPs and PCBs) and pathogens, or by acting at cellular or subcellular level (Velzeboer et al. 2014, Nelms et al. 2016, Jovanović et al. 2018, Critchell & Hoogenboom 2018, Foley et al. 2018).

Due to successful application of the optimised enzymatic digestion protocol in marine turtles to confirm the presence and ingestion of suspected microplastics and other synthetic materials, we recommend this protocol for surveying other large marine vertebrate gut content or to be used in combination with other novel techniques newly proposed in the literature (Caron et al. 2018, Felsing et al. 2018, Herrera et al. 2018). The method has already been used to demonstrate the presence of microplastic ingestion in marine mammals (Nelms et al. 2018). When there is clear overlap between high levels of microplastic pollution and the presence of large marine vertebrates, the application of this technique could aid in the confirmation of this occurrence and whether overlap results in ingestion, and with careful work, at what magnitude. Similarly the enzymatic digestion technique could be built into existing bioindicator protocols, which investigate macroplastic pollution, such as the Fulmar protocol (van Franeker & Law, 2015) and as such marine megavertebrates could serve as a bio-indicators for both macro- and microplastics.

By adapting a methodology previously used on marine invertebrates, this study has revealed that marine turtles are interacting with this cryptic pollutant. Further research is required to help discern which microplastic ingestion pathways are significant and whether there are species and site-specific variability in abundance and makeup of the particles ingested. Whilst these particles may be ubiquitous, and at higher levels than in marine mammals thus far surveyed, unless they play a role in amplifying exposure to associated contaminants, we suggest they are unlikely to present a significant conservation problem at current levels and are less of a concern than fisheries bycatch, the ingestion of macroplastics, or entanglement in anthropogenic marine debris (Nelms et al. 2016, Duncan et al. 2017).

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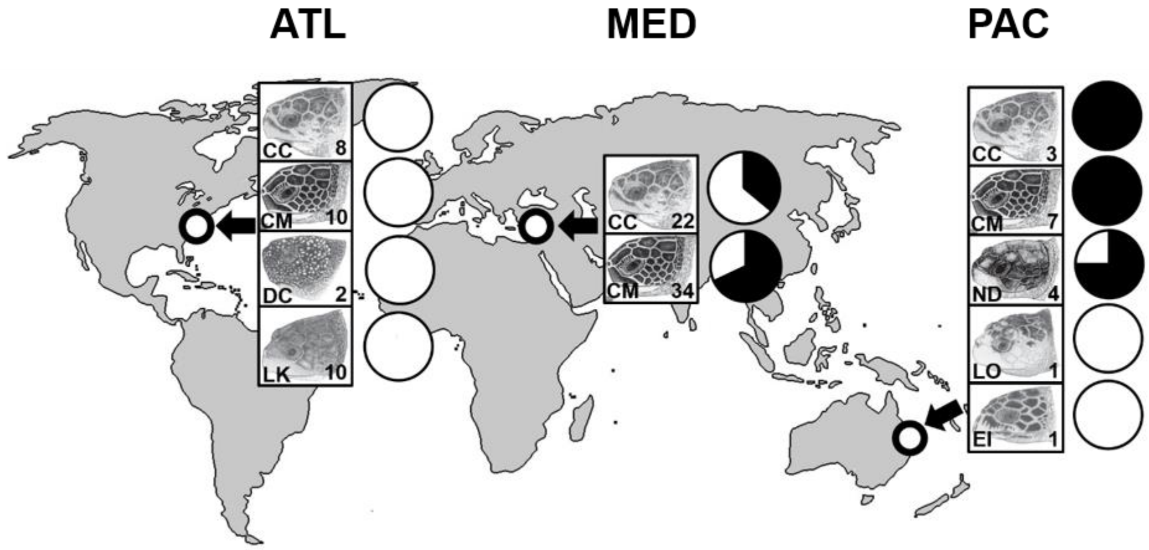

Figure 1. Study sites and number of each species sampled; Embedded pie charts of proportion of individuals with macroplastic ingestion (%); white=absent, black=present. Left to right: Atlantic (North Carolina, USA), Mediterranean (Northern Cyprus), Pacific (Queensland, Australia). Species codes: CC= loggerhead turtle (*Caretta caretta*), CM= green turtle (*Chelonia mydas*), DC= leatherback turtle (*Dermochelys coriacea*), LK= Kemp's ridley turtle (*Lepidochelys kempii*). ND= flatback turtle (*Natator depressus)*, EI= hawksbill turtle (*Ertmochelys imbricata*) and LO= olive ridley turtle (*Lepidochelys olivacea).* Sea turtle skull figures used with permission of WIDECAST; original artwork by Tom McFarland.

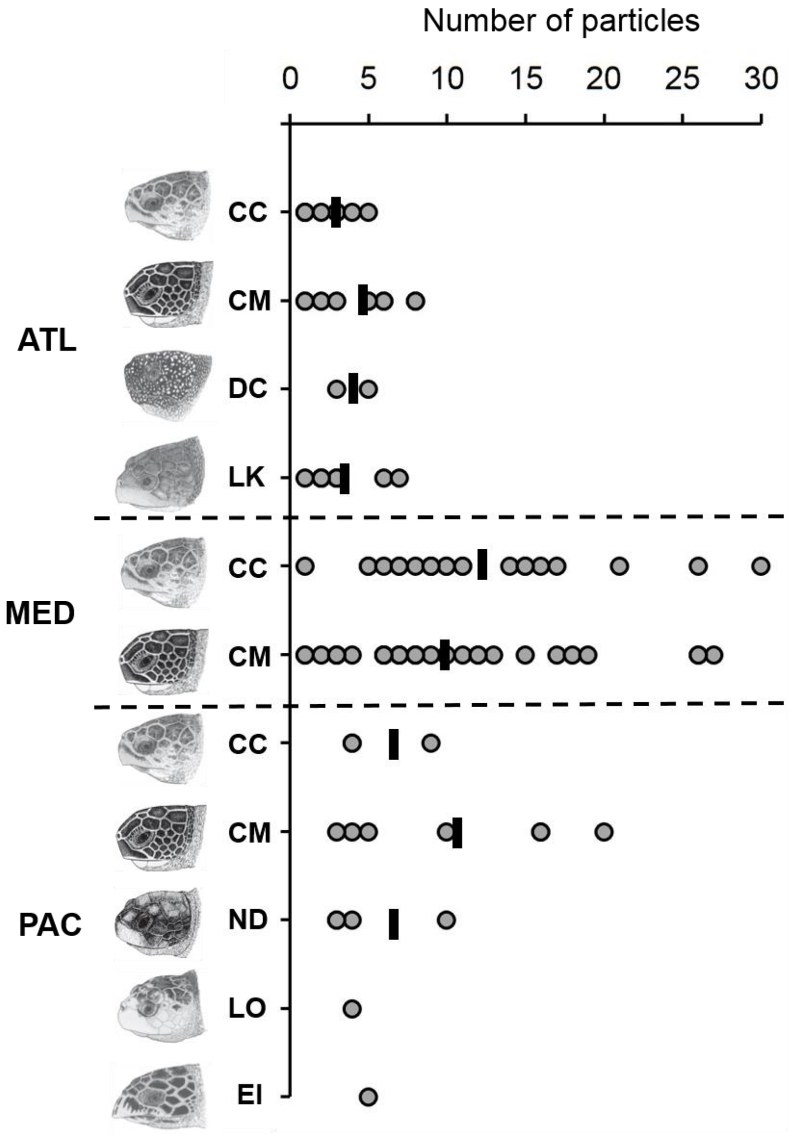
Number of particles
0
5
10
15
20
25
30
ATL
CC
CM
DC
LK
MED
CC
CM
PAC
CC
CM
ND
LO
EI

Figure 2. Synthetic micro-particle ingestion in all species of marine turtles from three ocean basins. Total number of particles identified in each sample per species per ocean basin. Black line =mean number of particles. Note that 100ml was analysed per animal irrespective of size, so the number of particles per animal should not be over-interpreted. ATL= Atlantic (North Carolina, USA) loggerhead turtle (*Caretta caretta*, n=8), green turtle (*Chelonia mydas,* n=10), leatherback turtle (*Dermochelys coriacea,* n=2), kemp's ridley turtle (*Lepidochelys kempii* n=10). MED= Mediterranean (Northern Cyprus) loggerhead turtle (n=22), green turtle (n=34). PAC= Pacific (Queensland, Australia) loggerhead turtle (n=3), green turtle (n=7), flatback turtle (*Natator depressus,* n=4), hawksbill turtle (*Eretmochelys imbricata,* n=1) and olive ridley turtle (*Lepidochelys olivacea,* n=1). Sea turtle skull figures used with permission of WIDECAST; original artwork by Tom McFarland

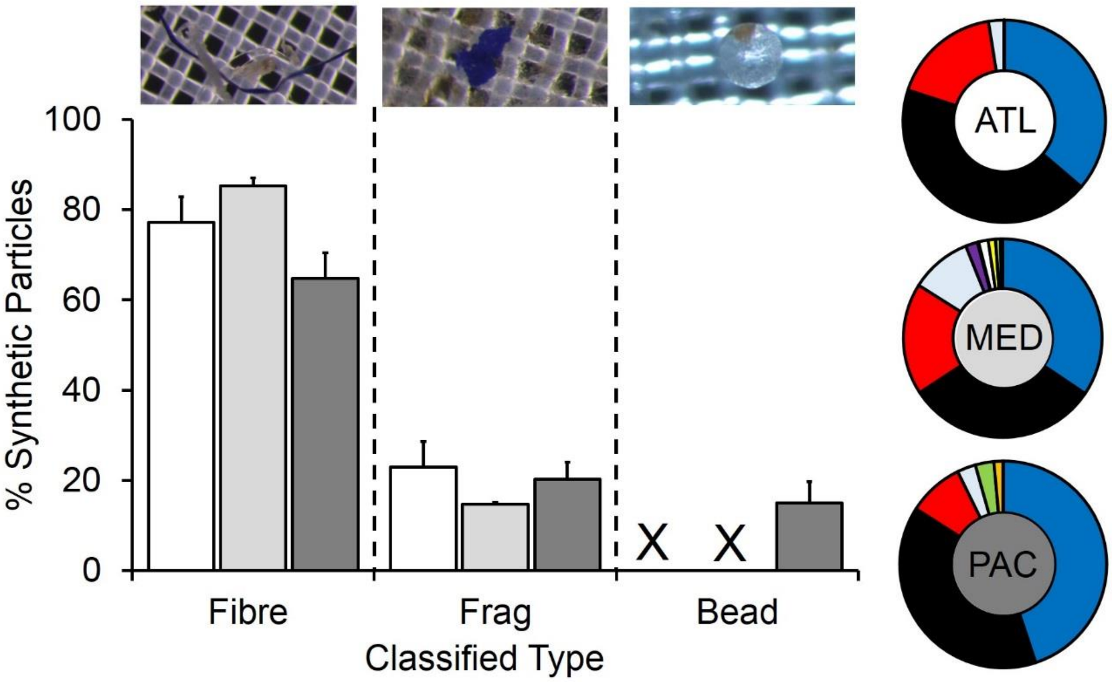

Figure 3. Type and colour of synthetic particles including microplastics identified from marine turtle gut content. Mean (±S.E.) percentage make up of each type (fibre, fragments, beads) isolated within the gut content residue samples from stranded turtles from the Atlantic (white), Mediterranean (light grey) and Pacific (dark grey). Colours categorised for fibrous synthetic particles ATL=Atlantic, MED= Mediterranean and PAC=Pacific. X= no-detections

Supplemental Methods

Necropsy and gut content analysis

Turtles were subject to necropsy to determine the cause of death, and biometric parameters were taken (Wyneken, 2001). To determine marine litter ingestion we followed the Fulmar Protocol developed by van Franeker et al., (2011) for monitoring plastic ingestion in the seabird *F. glacialis* which has been recommended to be adapted to the Mediterranean loggerhead turtle by the Marine Strategy Framework Directive GES Technical Subgroup on Marine Litter (Matiddi et al., 2011). During necropsy the entire gastrointestinal tract was removed and initial contents was weighed and then rinsed through a 1mm mesh sieve. After this, the remaining matter in the sieve was emptied into trays for sorting. Dietary items were separated, weighed and identified, meanwhile suspected plastic or other marine debris was removed and stored for later analysis. A sample of 100ml of gut content residue and was collected from material that had passed through the 1mm mesh sieve. This approximated 5% of the supernatant liquid. This was later oven dried at 60˚c for 24 hours to enhance the efficacy of homogenizing the remaining biological material in later steps of the process.

Enzymatic digestion

The optimised enzymatic digestion protocol was developed for use on zooplankton material by Cole et al., (2014) and adapted for use on marine turtle gut content. Desiccated samples were lightly ground with a pestle and mortar, to increase surface area, and transferred into 50mL acid-washed, screw-top glass containers (to avoid contamination) with 15ml homogenizing solution (400mM Tris-HCI buffer, 60mM EDTA, 105mM NaCl, 1% SDS). Samples were homogenized physically by drawing and expelling the mixture through a 19G needle attached to a 10mL syringe, the insides of which were rinsed thoroughly with homogenizing solution to avoid the loss of any material. Samples were then incubated at 50˚C for 30 minutes before adding 375µl of 20mg/mL^{-1} of Proteinase-K. These were further incubated for 2.5 hours at 50˚C and 3ml 5 M sodium perchlorate ($NaCLO_4$) was then added and samples shaken at room temperature for 30 minutes. Samples were homogenized a second time using a finer 21G needle, incubated at 60 ˚C for 30 minutes and then vacuum filtered on to pre-weighed 50µm mesh filters. Retained biological material was flushed copiously with Milli-Q water and the filters removed, covered and oven dried at 60 ˚C. To compensate for a greater amount of biological material having to undergo digestion from some gut content residue samples, filters were re-digested up to three times and each sample split between two to three 50 µm mesh-filters to prevent clogging and to more easily identify any microplastics present in these samples with higher amounts of biological material.

Filter analysis

Filters were analysed under a digital stereo microscope (Leica M165C). Microplastics particles were identified by assessing colour, uniformity of material and shape (Norén, 2007). These were then classified into three categories; fibres, fragments and bead. Microplastics were then further subcategorised into 11 colour categories (Black, Brown, Grey, White, Clear, Red, Orange, Yellow, Green, Blue, Purple). Particles were also measured; the length and width of fibres and the smallest diameter of fragments and beads, with examples photographed by a digital camera (Leica DFC295; Leica Suite Application Version 3.6.0).

Reducing contamination

A number of measures were implemented throughout the procedure to limit the risk of contamination of the samples via air-borne particles or is present on equipment: sterile containers were used for sample collection, all apparatus used within the laboratory was acid-washed and/or rinsed thoroughly with Milli-Q before use (filtered to ensure to be particle free). Personal protective equipment (e.g. cotton lab coat/ nitrile gloves) was worn at all times and samples and all surfaces were wiped down with 70% ethanol prior to any work commencing. Work (e.g. vacuum pumping) was carried out inside a positive pressure laminar flow hood and equipment were covered wherever possible to minimize periods of exposure with the aim of preventing air-borne microplastics from settling on the samples. During enzymatic digestion all equipment was rinsed with Milli-Q and all pipettes and syringes were flushed with Milli-Q prior to use. Furthermore, procedural blanks, from which gut residue material was omitted, were run in parallel from the initial sampling at gut processing of the marine turtles and through the enzymatic digestion process. Three blank samples were performed alongside each digestion process of gut content material, for each round of sampling in each field site (ATL n=3; MED n= 6; PAC= 3) and treated in the same way as samples to help check for possible contamination. The analysis of these filters (n=12) showed minimal evidence of microplastic contamination with the presence of single fibres (n=9 cases) or very occasional fragments (n=3 cases) but no beads. These particles were noted to look qualitatively different to those on the gut content filters i.e. environmental contaminants presented in full vivid colour wheras the ones from gut content were visibly degraded with faded colours.

Polymer Identification

The polymer make-up of marine plastic debris may aid in identifying possible sources, degradation, fate and reasons for ingestion (Jung et al. 2018, Nelms et al.

2018). A sub sample (n=169) of these identified microplastics were analysed using Fourier Transform Infrared spectroscopy (FT-IR) (Agilent Cary 630 FTIR spectrometer; Agilent FTIR Spectral Library ePoly 8; PerkinElmer Spotlight 400 FT-IR Imaging System, MCT detector, KBr window; PerkinElmer Spectrum software version 10.5.4.738) to determine their polymer make up. When interpreting FTIR output, only match qualities greater than 70% or greater and those considered to have reliable spectra matches (after visual inspection) were accepted.

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Site	Species	n	CCL range (cm)	Date Range	Macro-plastic ingestion (%)	Synthetic μ particle Total no.				
						Elastomers	Woven	Plastics	SCRFs	Non-Syn.
MED Northern Cyprus (Eastern Mediterranean)	Green	34	25-86	2011-16	68	22	2	12	3	3
	Loggerhead	22	12 77	2011-16	36	52	4	13	4	6
ALT North Carolina, USA (Eastern Atlantic)	Green	10	25-35	2016-17	30	0	0	4	2	0
	Loggerhead	8	55-83	2016-17	0	0	0	1	4	0
	Kemp's Ridley	10	23-41	2010-17	0	0	0	1	4	0
	Leatherback	2	148-U	2017	0	0	0	1	2	0
PAC Queensland, Australia (Coral Sea, Pacific)	Green	7	6- 57	1993-2017	100	0	0	4	11	0
	Loggerhead	3	5-7l	2009-14	100	0	0	2	3	0
	Flatback	4	10-23	2006-14	75	1	0	2	3	0
	Olive Ridley	1	61	2016	0	0	0	0	1	0
	Hawksbill	1	59	2016	0	0	0	0	2	0

Table S1. Summary of marine turtles (n=102) by sites, species, size (CCL: Curved Carapace Length cm; notch to notch), % macroplastic and synthetic particle ingestion presence. U=unmeasured due to damage

Origin	**Group**	**FT-IR Identification**	**MED n= 121**	**ATL n= 19**	**PAC n= 29**
Synthetic	Elastomers	Chlorobutyl-536 Blair	1	-	-
		Chlorobutyl-1051 Polycorp	1	-	-
		Chlorobutyl-516 Blair	5	-	-
		Ethyl-acrylate Vamac (Rubber)	3	-	-
		Ethylene Propylene Diene Monomer (EPDM Rubber)	16	-	-
		Hydronated Nitrile Butadiene Rubber (HNBR)	19	-	-
		Nitrile-Butadiene Rubber (NBR)	11	-	1
		Ethylene Propylene	8	-	-
		Neoprene	7	-	-
		Viton	3	-	-
			61.2%	0%	3.4%
	Woven	Aramid Woven Fabric	3	-	-
		Polyaramid, Kevlar® woven fibers	3	-	-
			4.9%	0%	0%
	Plastics e.g. thermoplastics	Klockner Moeller 74 Relay Housing Piece2	1	-	-
		Nylon	-	2	-
		Paraffin Wax and Polyvinyl Acetate Mixture	2	-	-
		Polyacrylamide,Carboxy modified	3	1	2
		Polyacrylic	-	2	1
		Polyacrylate	2	-	-
		Polycarbonate	-	1	-
		Polyester Fibers	4	-	3
		Polyethylene terephthalate	6	-	-
		Polyethylene, chlorinated	7	-	1
		Polypropylene	-	-	1
		Plastised Polyvinyl Chloride (PVC)	-	1	-
			20.6%	36.8%	27.9%
	Regenerated Cellulose	e.g. Rayon or Viscose	7	12	20
			5.8%	63.2%	68.9%
Non-synthetic	Rubbers	Natural Latex Rubber	2	-	-
		Natural Rubber	4	-	-
	Other	Zein	3	-	-
			7.4%	0%	0%
Total:			**121**	**19**	**29**

Table S2. Results from the subsample of isolated particles (n=169) analysed using Fourier transform infrared spectroscopy (FT-IR) to determine their polymer make up from gut content residue samples of marine turtles

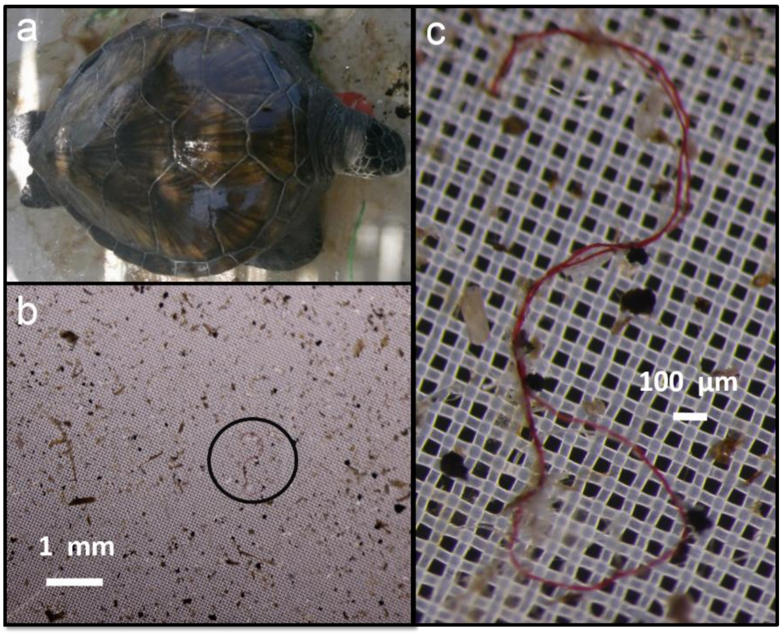

Figure S1. Enzymatic digestion of marine turtle gut content a) Stranded juvenile green turtle (CCL=33cm) from the North Cyprus coastline b) the gut content residue sample from the juvenile green turtle that has been enzymatically digested which has removed the majority of the biological material allowing the identification of suspected microplastics c) a microplastic fibre isolated from the gut content of the juvenile green turtle.

Chapter 6. The True Depth of the Mediterranean Plastic Problem: Extreme Microplastic Pollution on Marine Turtle Nesting Beaches in Cyprus

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Abstract

We sampled 17 nesting sites for loggerhead (*Caretta caretta*) and green turtles (*Chelonia mydas*) in Cyprus*.* Microplastics (<5 mm) were found at all locations and depths, with particularly high abundance in superficial sand. The top 2cm of sand presented grand mean±SD particle counts of 45,497±11,456 particles.m^{-3} (range 637-131,939 particles.m^{-3}). The most polluted beaches were among the worst thus far recorded, presenting levels approaching those previously recorded in Guangdong, South China. Microplastics decreased with increasing sand depth but were present down to turtle nest depths of 60cm (mean 5,325 ± 3,663 particles.m^{-3}. Composition varied among beaches but hard fragments (46.5±3.5%) and pre-production nurdles (47.8±4.5%) comprised most categorised pieces. Particle drifter analysis hindcast for 365 days indicated that most plastic likely originated from the eastern Mediterranean basin. Worsening microplastic abundance could result in anthropogenically altered life history parameters such as hatching success and sex ratios in marine turtles.

Introduction

Plastic in the Marine Environment

Plastic is now ubiquitous in the marine environment and accounts for 86% of all anthropogenic marine debris globally (Laist 1987, Barnes et al. 2009, Ivar do Sul & Costa 2014, Jambeck et al. 2015, Nelms et al. 2017). Its mobility and high concentrations allow it to interact with a wide variety of marine biota through multiple pathways, and so plastic is considered a growing threat to marine biodiversity (Derraik 2002, Cole et al. 2013, Gall & Thompson 2015, Nelms et al. 2016). The dispersion of plastic across oceans facilitates the rafting of invasive species, plastic entanglement and ingestion, causing injury and death (Derraik 2002, Gall & Thompson 2015, Nelms et al. 2016, Duncan et al. 2017).

Microplastics

By definition, microplastics (<5 mm) (Andrady 2011) can enter the marine environment from primary sources via industrial spills as pre-production nurdles, through runoff from sewage systems, as microbeads from cosmetics, and as microfibers from clothes or tyre wear (Moreira et al. 2016, Nelms et al. 2017, Gago et al. 2018). Microplastics can also be created secondarily through fragmentation, whereby discarded macroplastics (≥5 mm) breakdown through UV exposure and mechanical abrasion, such as wave action and weathering (Hopewell et al. 2009, Andrady 2011). As fragmentation continues particle size reduces; for example the mean length of plastic in the North Atlantic reduced from 10 mm to 5 mm between 1991-2017 (Morét-Ferguson et al. 2010).

The scale of the problem mandates a focus on the biological impacts of microplastics (Ivar do Sul & Costa 2014, Vegter et al. 2014, Nelms et al. 2016). This includes assessing their ability to be passed up the food chain through trophic transfer (Fossi et al. 2012, Cole et al. 2013, Van Cauwenberghe et al. 2015). Additionally, the affinity of plastics with PCBs and other toxic chemicals, enables microplastics to be a potential vector for the trophic transfer of toxins (Ryan et al. 1988, Tanaka et al. 2012, Storelli & Zizzo 2014).

Microplastics & Beach Sediments

Microplastic abundance on beaches is thought to have tripled over the last twenty years (Moore 2008, Ivar do Sul & Costa 2014). Microplastics wash onto beaches from surface waters and become incorporated within the sediment as beach volumes alter through erosion and accretion events (Thom & Hall 1991, Barnes et al. 2009, Poeta et al. 2014). In contrast with natural sediments, microplastics are more angular, resulting in unpredictable patterns of weathering (Cooper & Corcoran 2010). These atypical properties have been shown to have the potential to increase sediment permeability and porosity, and decrease substrate temperatures (Carson et al. 2011). However other studies consider that temperatures would increase as plastics have a higher specific heat capacity than sand, especially if the pigment of the plastic is dark (Andrady 2011, Beckwith & Fuentes 2018). Marine turtle nesting success is strongly influenced by extrinsic factors during egg development (McGehee 1990, Ackerman 2002, Warner 2014). In particular, temperature influences the duration and success of development and determines the sex of offspring (Ackerman 2002, Horne et al. 2014, Hays et al. 2017). High microplastic abundance within sand in turtle nests could impact hatching success and skew hatchling sex ratios (Cooper & Corcoran 2010, Nelms et al. 2016).

Microplastics & Mediterranean Marine Turtles

Northern Cyprus hosts some of the most important nesting beaches in the Mediterranean for both loggerhead (*Caretta caretta*) and green turtles (*Chelonia mydas*) (Kasparek et al. 2001, Broderick et al. 2002, Stokes et al. 2015). The Mediterranean basin is associated with dense coastal populations with high levels of anthropogenic waste and variable governance levels (Coll et al. 2010), consequently the Mediterranean has been found to hold plastic concentrations comparable to the largest congregations of plastic on the globe such as in the North Pacific gyre e.g. $>10^5$ particles km^{-2} (Cózar et al. 2014, 2015, van Sebille et al. 2015). This study aimed to: 1) quantify the composition, distribution, abundance and spatial variation of microplastics across beaches in Cyprus 2) look at how this varied at depth in the sediment and 3) use oceanographic current models to identify the potential source locations of the plastic.

Materials & Methods

Study Area

Sampling was carried out at 17 beaches along the coastline of Cyprus in the Eastern Mediterranean between July and August 2016 (Figure 1; Supplemental Table 1). Surveys were coincided with the main period of turtle nesting/hatching activity. Beaches were selected, based upon their spatial distribution and high turtle nesting densities (Broderick et al. 2002).

Sediment Sampling

Within each beach, sediment samples were collected from 10 pairs of sampling sites along two lines parallel to the shore: the “strandline” (SL) and the “turtle nesting line” (TNL). The 10 sampling sites were spaced equidistantly along the beach length, avoiding rocky edges of the beach (Supplemental material Figure 1). Co-ordinates were taken at all sample locations (longitude/latitude: World Geodetic System (WGS) 1984 format) using a Garmin eTrex® 10 handheld GPS device. (Supplemental Table 1.) Strandline (SL) was defined as the highest line of debris left from the retreating tide. This meandering line where debris accumulates is periodically generated by tide, wave and air movements (Heo et al. 2013). The turtle nesting line (TNL) was a transect through typical turtle nesting area. This was approximately the medial distance between strandline and the landward limit of the beach within which turtles nested, approximated by a) marked nests recorded as part of exhaustive ongoing monitoring, b) body pits left from nesting attempts (Broderick & Godley 1996).

All samples were collected using a bespoke cylindrical galvanised steel corer of 20cm diameter and 60cm height. A volume of 250cm^3 was gathered for 0-2cm depth at sampling locations on the strandline (SL) to allow for comparisons with recent similar studies (e.g. Clunies-Ross *et al.* 2016; Yu *et al.* 2016; Zhang *et al.* 2016). At the turtle nesting line (TNL) a volume of 250cm^3 was taken from incremental depths (0-2.0, 2.1-10.0, 10.1-20.0, 20.1-30.0, 30.1-40.0, 40.1-50.0, 50.1-60.0cm). Due to striking water or rock it was not always possible to core to the full 60cm. Samples were air dried in metal trays covered in aluminium foil to avoid loss and/or contamination of microplastics from other environmental sources prior to processing.

Separation and Categorisation

Dry weight of whole sediment subsamples was measured to an accuracy of 0.01g, before being passed through a sieve cascade of 5 mm and 1 mm to capture microplastics (<5 mm and >1 mm (Andrady 2011). Anthropogenic debris was then isolated from each sample and categorised based on procedures proposed by van Franeker *et al.* (2011).

Plastic categories

Plastics were then assigned to one of five categories (van Franeker et al. 2011): (1) Industrial (IND) – Roughly spherical plastic pellets used in industrial practice as primary pre-production material to melt and mould (known as: nurdles, pellets, beads, granules); (2) Foamed (FOAM) – Synthetic sponge, mattress foam, polystyrene, polyurethane; (3) Fragment (FRAG) – Broken down pieces of hard plastic from bottles and other consumer items; (4) Sheet-like (SHE) – remains of sheeting and bags; and (5) Thread-like (THR) - remains of netting, ropes, net packaging, nylon fishing line**.** Microplastic debris from each category within each sample was counted and weighed to 0.0001g. With these data, dry weights and known volume data were converted into four different units for analysis and comparison with the wider literature: particles.m^{-3}, particles.g^{-1}, g.m^{-3} and g.g^{-1}.

Particle Drifter Analysis

To investigate the potential source and at-sea trajectories of floating, passive plastic we used the Parcels framework (Lange & van Sebille 2017) to model backward trajectory probabilities for virtual particles released from seventeen beaches (Supplemental Table 2.). Using established methodologies from Lagrangian Ocean Analysis (van Sebille et al. 2018), the virtual particles were transported by the flow from hydrodynamic circulation models. Hydrodynamic data were sourced from the HYbrid Coordinate Ocean Model (HYCOM: hycom.org) + NCODA Global Reanalysis at 1/12 degree resolution and daily output frequency (Cummings & Smedstad 2013). One particle was released from each beach for every day from 5 July 2015 to 1 July

2016 with each particle being advected (back in time) for 365 days. The time-step of the 4th order Runge-Kutta integration was 5 minutes and particle locations were saved at daily frequency. Due to spatial limitations within the HYCOM gridded data, start locations for back-tracked drifter simulations from beaches 15, 16 and 17 (Figure 1.) were relocated 0.06 degrees east (approx. 5 km) to enable flow to be simulated around these release sites. The python code for these simulations is available at https://github.com/OceanParcels/Plastic_CyprusBeaches/.

For each beach release location, a sampling grid of 20 x 20 km grid squares was used to sum all spatially coincident daily drifter trajectory locations. The same sampling grid was used to determine the number of individual drifter trajectories traversing a grid square. To enable 'at sea' trajectories to be clearly displayed, trajectory location data within 5 km of the coast were removed from the analysis. Where back-tracked particle trajectories terminated at coastal locations (particles became stationary and were no longer advected) these were deemed to be the source location for the trajectory and were summarised by country.

Results

Overview

A total of 1,209 sediment samples were obtained from 170 turtle nesting area samples and 170 strandline sampling locations across the 17 nesting beaches. Microplasticswere found to be pervasive in all sampled locations and depths, with particularly high abundance within the top 2 cm of sand. The grand mean of microplastics in surface samples in the TNL (turtle nesting line) was 45,497 ± 11,456 (mean ±se) particles.m^{-3} (range across 17 beaches: 637-131,939 particles.m^{-3}) and a grand mean weight of 481 ± 131 g.m^{-3} (range across beaches: 1 - 1,714 g.m^{-3}).

There was no significant difference between mean values on the strandline and the turtle nesting line (Paired t-test: particles.m^{-3} t_{16}= 1.14, p= 0.28; g.m^{-3} : t_{16}= 0.07, p= 0.94; Supplemental Table 1).

Beach Variation

Abundance of microplastics in the turtle nesting line was found to vary significantly across beaches in both particles (particles.m^{-3}; ANOVA, $F_{2,14}$=12.32, p < 0.001) and mass (g.m^{-3}; ANOVA, $F_{2,14}$=13.52, p < 0.001). Coastal position of the beach had a significant effect on microplastic abundance (particles.m^{-3}: $F_{2,14}$= 11.42, p <0.001; g.m^{-3} $F_{2,14}$= 13.97,p <0.001) with significantly higher levels on the North Coast compared to both the West and East coasts: particles.m^{-3} (Tukey's Honest Significant Difference, North > West: p<0.001; North > East: p< 0.001; West = East: p= 0.95), g.m^{-3} (Tukey's Honest Significant Difference, North > West: p=0.01; North > East: p< 0.001; West = East: p= 0.97). The highest microplastic abundances of 131,939 ± 34,000 particles.m^{-3} occurred on Beach 10 (North Coast) (Figure 1.; Supplemental Figure S2.)

The grand mean maximum depth reached by core samples was 49.5 ± 1.2cm however, maximum depths reached varied considerably by core (range = 8 - 60cm) with 116 complete cores sampled. Microplastics were found at all depths within sampled beaches, with particles discovered down to 51-60cm with mean levels of 5,325 ± 3,663 particles.m^{-3} and 59 ± 39 g.m^{-3} (range: 381 - 63,344 particles.m^{-3}; 4 - 638 g.m^{-3}) at that depth. (Figure 2.Supplemental Figure S3). This difference among depths was found to be significant for both particles.m $^{-3}$ (Kruskal-Wallis test, $\chi^2(6)$ = 28.32, p <0.001) and g.m^{-3} (Kruskal-Wallis test, $\chi^2(6)$ = 23.06, p <0.001); with more microplastics found at shallower levels (Figure 2. Supplemental Figure S3). Of the five plastic categories, industrial (IND) and fragment (FRAG) made up >85% of microplastic particles present in samples per volume (decreasing in abundance in FRAG>IND>FOAM>SHE>THR) and 98% by mass (IND>FRAG>SHE>FOAM>THR) (Figure 3.).

Particle Drifter Analysis

Hindcast modelling of at-sea trajectories of plastic revealed that the major source locations occurred almost exclusively in the eastern part of the Mediterranean basin with limited counts from the western section of the basin e.g. Italy, Malta and Tunisia (Figure 4; Supplemental Figure S4). There was variability in the count of particles tracked to each drifter source location, with most modelled particles making landfall elsewhere in Cyprus, Turkey and Lebanon and dense particle presence in off-shore accumulation zones (Figure 5).

Discussion

Microplastics at Depth

The ubiquitous nature of microplastics within nesting beach environments, supports the idea that beaches act as microplastic sinks for the wider oceans (Barnes et al. 2009, Poeta et al. 2014, Nelms et al. 2016) becoming key areas of environmental contamination. Levels in Cyprus were 5-1000 times higher in comparison to other regional studies from Greece, Malta and Spain (Turner & Holmes 2011, Kaberi et al. 2013, Alomar et al. 2016) and orders of magnitude higher than surface levels on marine turtle nesting beaches in Florida, USA (Beckwith & Fuentes 2018). Indeed, upon reviewing the literature, the levels of microplastics present on beaches in Cyprus were among the worst thus far recorded, presenting abundances approaching those previously were recorded in Guangdong, South China in 2015 (166,875 ± 175,525 particles.m^{-3}; range of means across 8 beaches: 6,200-437,625 particles.m^{-3}) (Fok et al. 2017). Waste input between China and Cyprus however, varies markedly with China producing 27.8% of global plastic, 50% more than the whole of Europe (Plastics Europe 2016)), beaches in China are therefore likely to be contaminated from direct, local inputs (Tsang et al. 2017). In contrast many sample beaches in Cyprus are located far from industrial practices with little human usage, therefore likely receiving microplastic via ocean currents from around the eastern Mediterranean (Barnes et al. 2009) Our data are indicative of the generally high plastic levels found within the Mediterranean Sea (Cózar et al. 2015, van Sebille et al. 2015, Alomar et al. 2016).

Microplastics , the vast majority of which are likely to have come via the sea, were ubiquitous upon the beaches of northern Cyprus and were present down to nesting depths of loggerhead and green turtles (Broderick et al. 2002). The ability of significant amounts of small plastic particles to be transferred down through sediments corresponds with the few studies previously undertaken (Carson et al. 2011, Turra et al. 2014). Changes to the incubation environment for eggs could result as microplastics exhibit different physical properties to natural sediments, high abundances could potentially impact nesting success and skew hatchling sex ratios. Carson *et al.* (2011), used experimental sediment cores to show that higher microplastic abundance increased the permeability and decreased the temperature of sediment. However plastic values in their experimental cores (15.9-29.4% by weight) producing significant effects were very much higher than levels found in this study. Marine turtle eggs rely on the uptake of water during development, therefore increased permeability from high microplastic abundances has the potential to reduce nesting success through desiccation. Furthermore other studies argue that temperatures would increase with the presence of plastic (especially with dark pigments) as they have a higher specific heat capacity than sand (Andrady 2011, Beckwith & Fuentes 2018). Further experimental studies are clearly needed to evaluate the impact of plastic presence in the sand column on critical parameters such as temperature and permeability. Potential study ideas could include experimental “nests” that have been spiked with environmentally relevant plastic concentrations.

Among Beach Variation

Microplastic abundance varied among sampled beaches with significantly more microplastic was found upon the north coast compared to those of the west or east coast; the influence of current and wind patterns moving of particles around coastline (van Sebille et al. 2015). The Levantine Basin, in which Cyprus is situated, has very little interaction with the rest of the Mediterranean (Hecht et al. 1988). Plastic that enters the basin from surrounding countries (Egypt, Israel, Lebanon, Syria, and Turkey, Cyprus) is also washed up on the beaches of those countries (Mansui et al. 2015, Zambianchi et al. 2017). Hydrodynamic (current) influences were clearly demonstrated within the particle drifter models illustrating to the anticlockwise currents of the Levantine basin. It should be noted, however, the modelled source locations achieved from the model may not be the primary origin of the plastic debris but may be interim locations as plastic moves around the region via offshore accumulation zones. For instance plastic accumulates in the Shikmona anticyclone gyre (SMA), off the SE coast of Cyprus (Alhammoud et al. 2005, Cózar et al. 2015, Zambianchi et al. 2017). This plastic is then caught in the strong north-easterly current and carried up the east coast of Cyprus where it is then propelled westward before being deposited on the north coast (Alhammoud et al. 2005).

Variance among Plastic Categories

Microplastics sampled varied considerably in abundance between plastic categories (IND, FOAM, FRAG, SHE and THR). Fragments of harder plastics (FRAG) and industrial pellets (IND) making up the majority of the microplastic particles. These differences in migration, breakdown and deposition of different microplastic types may be explained by the re-suspension of sediments; the nature of fragments and rounded pellets behaving in a different way to films, flakes and fibres (Chubarenko & Stepanova 2017). Indeed modelling of microplastics in the marine environment has revealed that foamed plastics travel fastest over surface water and films and fibres typically sink due to higher rates of bio-fouling than fragments or spheres which could explain their lack of abundance upon beaches (Chubarenko et al. 2016).

Call for Standardisation

To better understand the distribution of anthropogenic waste globally, comparative studies are important however this requires standardisation within the field. For example macroplastic and beach litter standards recommendations have been developed by the TG Marine Litter working group, whose guidance covers methodologies and the harmonisation of protocols (Hanke 2016) . They have also refined tool kits for microlitter sampling in intertidal and subtidal sediments, working towards standard methods to sample shorelines, sea surface and seabed (MSFD GES Technical Subgroup on Marine Litter 2011). Current methodologies specifically for microplastic sampling still need a number of clarifications to achieve standards. Of priority requirement is a clear definition of ‘microplastic’. Whilst a majority of studies take the definition from Andrady (2011) microplastics are particles <5 mm in size, some modern studies use the upper boundary of 1 mm, more closely linked to the definition of ‘micro’ (Browne et al. 2007, Costa et al. 2010, Van Cauwenberghe et al. 2015). Using an upper limit of 1 mm fails to account for industrial pellets (IND) which have a mean size of 3-4 mm (van Franeker et al. 2011). These plastic particles are too small to fit into other larger plastic sampling, which usually cuts off at a bottle top size of *ca.*20 mm (OSPAR 2010)). As pellets remain significant in both abundance and ingestion, a practical proposal comes as the reclassification of microplastic into ‘large microplastic’, 1-5 mm and ‘small microplastics’, <1 mm (Van Cauwenberghe et al. 2015). This would account for both the importance of industrial and finer microscopic fibre filaments (Claessens et al. 2011, Turra et al. 2014). It would allow further neatening of the division between sampling techniques. ‘Large microplastic’ sampling following more accessible protocols, of sieving and categorisation by eye, as in this study. ‘Small microplastics’ adopting the refined techniques of particle floatation and microscopic identification (Hidalgo-Ruz et al. 2012).

Secondly we call for standardisation of units in sampling protocols. We noted at least seven different units used within beach sampling papers: particles m^{-2}, particles m^{-3}, particles g^{-1}, g m^{-2}, g m^{-3}, g g^{-1} and % of plastic by weight (Hidalgo-Ruz et al. 2012). We propose reporting data in particles.m^{-3} and g.m^{-3} for specific area, depth and volumes of sand. Additionally when considering standardisation it is also important to study the chemical characterisation of microplastics removed from beach sediments. Although outside the scope of this study it is becoming evident that obtaining the polymer make-up either by FT-IR or Raman Spectroscopy is highly beneficial for assessment of beach contamination and to understand potential impact (Jung et al. 2018), therefore standard methodologies should include this in their design.

Conclusion

The turtle nesting beaches of Cyprus are exposed to the highest published microplastic abundances within the Mediterranean, second globally only to Hong

Kong, China. The majority of microplastic found in our study originated from industrial spills, followed by fragments from the breakdown of larger plastic pieces. Standardised methodology for sampling microplastic in beach sediment will allow for more effective global comparisons and understanding the effects of this novel pollutant, a research priority for the taxon (Rees et al. 2016). This study highlights that, within the eastern Mediterranean, threats to turtle nesting ecology from microplastic; induced desiccation, toxicology and changes to hatchling sex ratios are possible in the future. Experimental studies of nest environments under variable and experimentally controlled microplastic density are clearly mandated.

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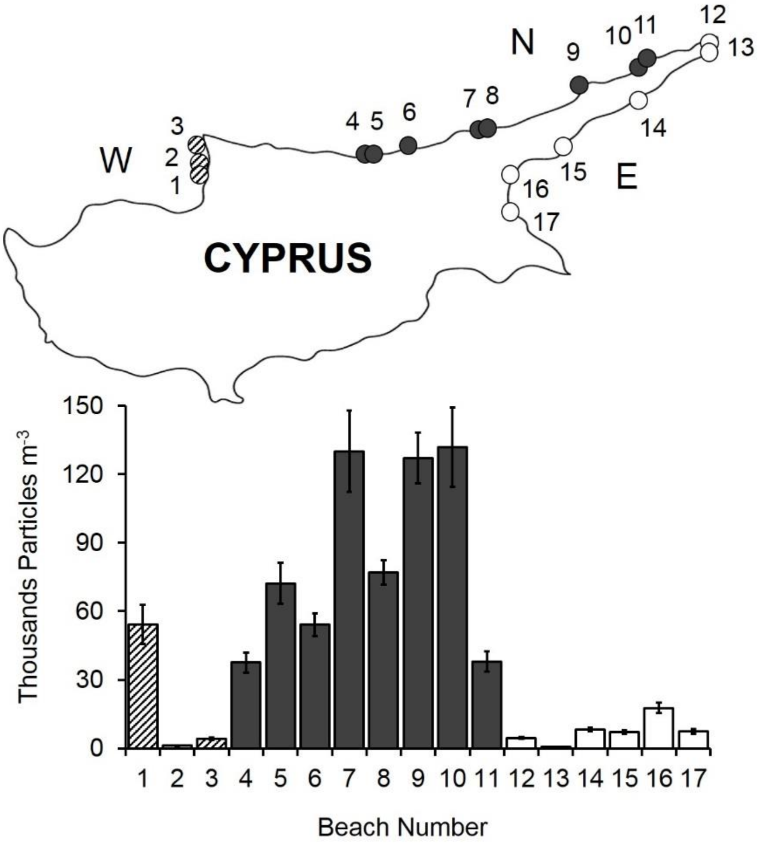

Figure 1. Mean microplastic in particles m^{-3} within turtle nesting line (TNL) surface samples (0-2cm), across numbered sample beaches with fitted standard error bars. Stack shades represent the three different coastlines in the map insert: Hatched= West (n=3, beach number 1-3), Grey = North (n=8, beach number 4-11), White = East (n=6, beach number 12-17). Individual beach co-ordinates can be found in Table 1, supplementary data.

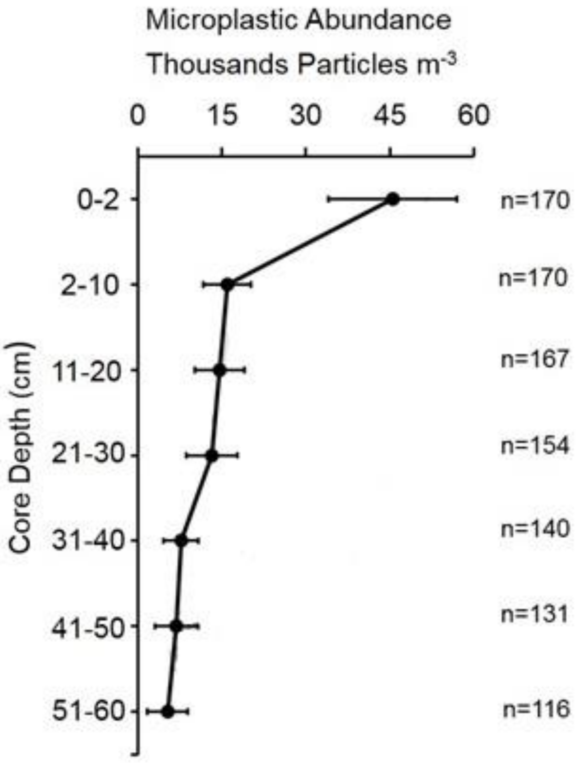

Figure 2. Grand mean (±S.E) of microplastic abundance in particles m^{-3} at different sand depths at turtle nesting areas (n=17 sites).

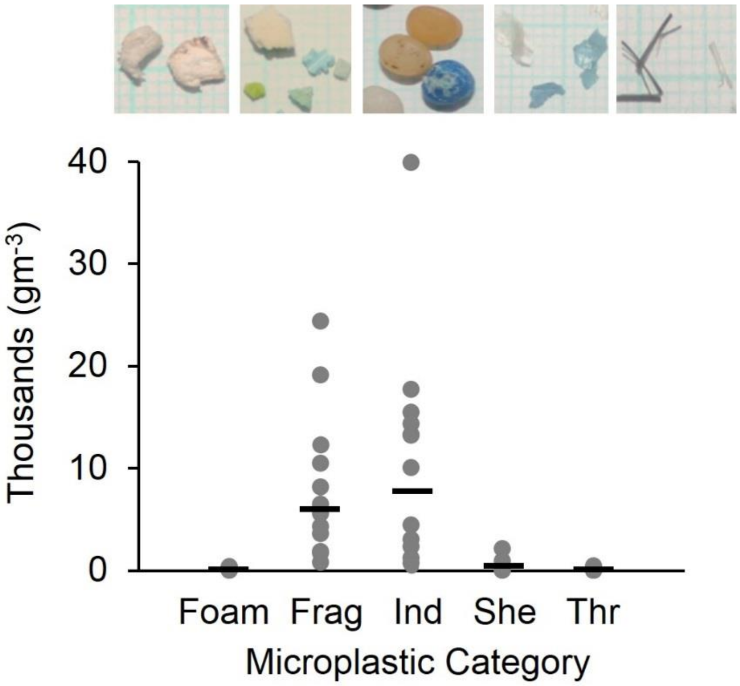

Figure 3. Microplastic weight/volume (g m^{-3}) classification categories on each beach (grey dots) (n=17). Black line = mean microplastic weight/volume (g m^{-3}) across all sample beaches cores.

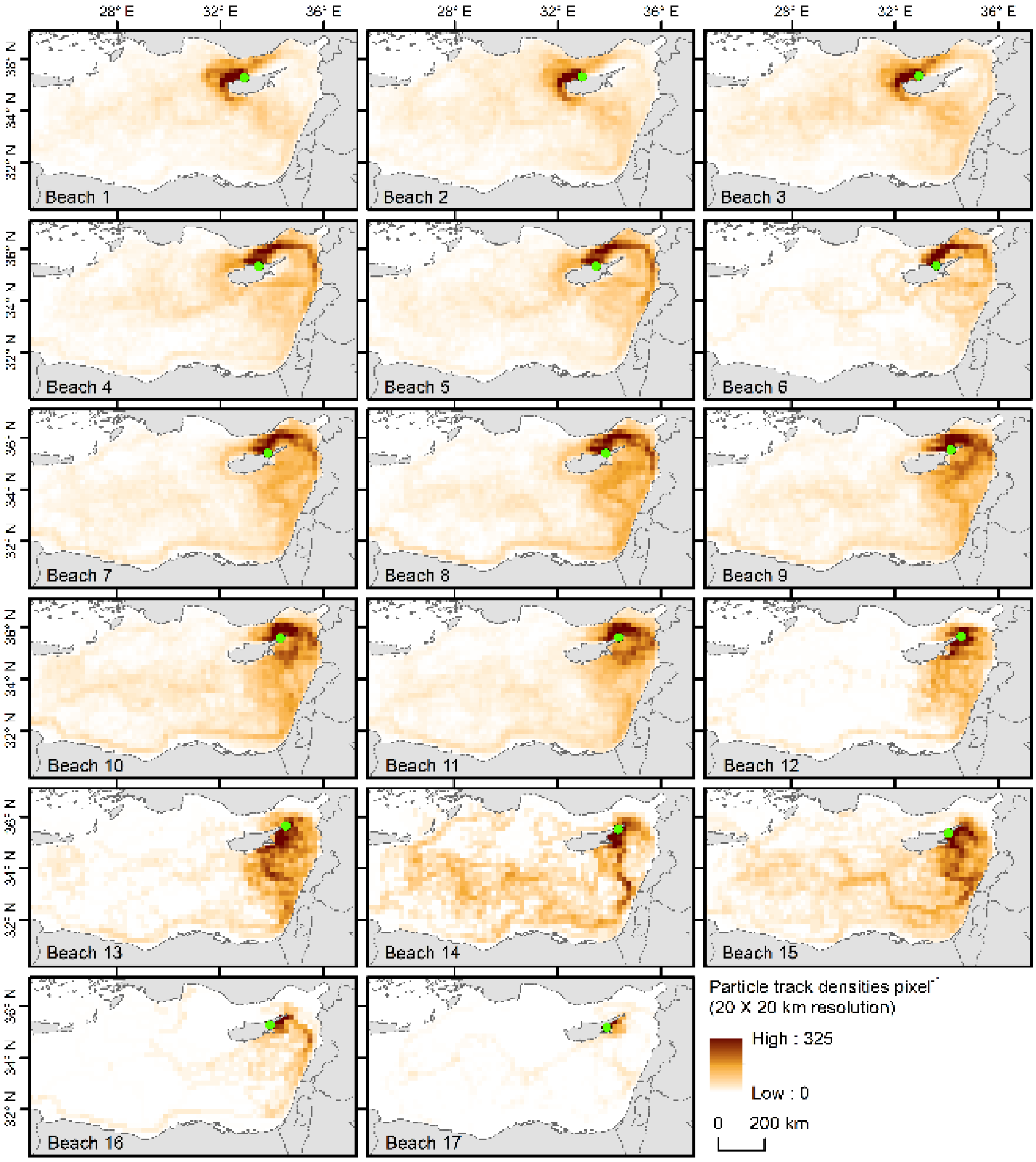

Figure 4. Particle trajectories (mapped by receiving beach; n=17) rasterised to a 20 x 20 km grid resolution. Tracks per grid square are counted. To enable 'at sea' trajectories to be clearly displayed data within 5 km of the coast have been removed.

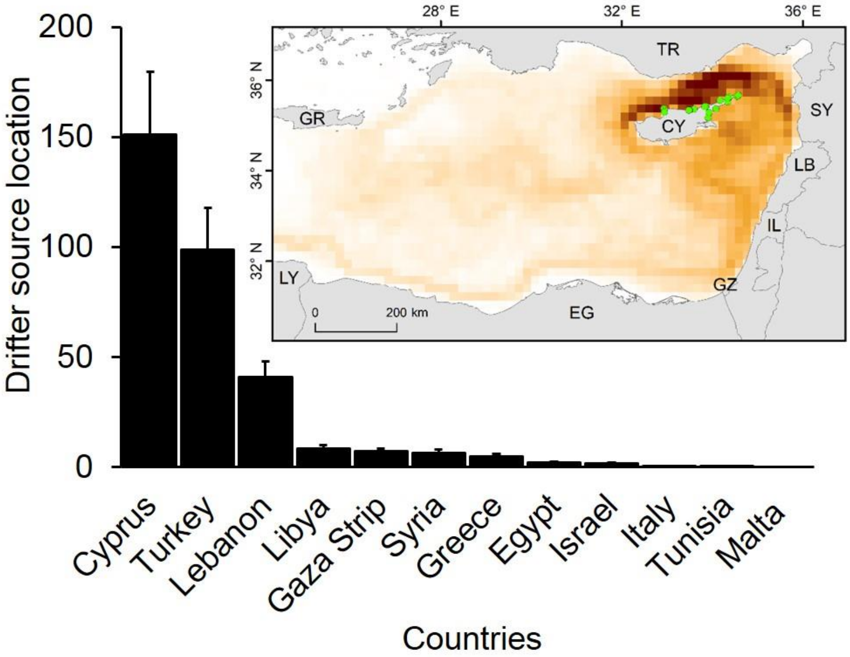

Figure 5. Drifter source locations (mean ± s.e.) by country for monitored beaches (n=17). Countries are identified using their 2 digit sovereign state ISO code as follows: Greece (GR), Turkey (TR), Cyprus (CY), Syria (SY), Lebanon (LB), Israel (IL), Gaza Strip (GZ), Egypt (EG) and Libya (LY).

Chapter 6; Supplementary Information

Beach Number	**Beach Coordinates**	**Strandline (SL)**		**Turtle Nesting Line (TNL)**	
		Mean Particles m³	Mean gm³	Mean Particles m³	Mean gm³
1	35.29311N 32.93944E	333748	602	54272	177
2	35.32631N 32.93527E	96607	634	1114	9
3	35.36705N 32.92333E	37720	328	4138	86
4	35.33255N 33.48277E	28489	350	37561	498
5	35.33463N 33.49305E	79577	782	72256	697
6	35.35416N 33.59750E	24987	134	54113	711
7	35.41191N 33.83416E	50452	290	130030	1115
8	35.41592N 33.86361E	47428	302	76872	959
9	35.54833N 34.17166E	197352	1600	127165	1714
10	35.60072N 34.33388E	28330	172	131939	1438
11	35.62633N 34.36972E	23077	110	38038	348
12	35.66666N 34.57222E	1909	35	4456	151
13	35.64116N 34.54694E	60638	172	637	1
14	35.52297N 34.33972E	21963	108	8117	95
15	35.36511N 34.07944E	9231	55	8435	66
16	35.27869N 33.92500E	18144	45	17666	63
17	35.16805N 33.90944E	3024	34	6645	41

Table S1. Microplastic levels across study beaches (n=17). Co-ordinates presented in DMS (Degrees, Minutes, Seconds). Mean values in particles m^{-3} and $g.m^{-3}$ for the strandline (SL) and turtle nesting line (TNL).

Country	Drifter Source locations (Mean±SE)
Cyprus	151.0±28.6
Egypt	2.2±0.5
Gaza Strip	7.2±1.4
Greece	5.0±0.9
Israel	1.7±6.9
Italy	0.3±2.4
Lebanon	41.1±6.9
Libya	8.6±1.5
Malta	0.0±0.0
Syria	6.5±1.5
Tunisia	0.2±0.1
Turkey	98.9±18.9

Table S2. Drifter source locations (mean ± s.e.) by country for monitored beaches (n=17).

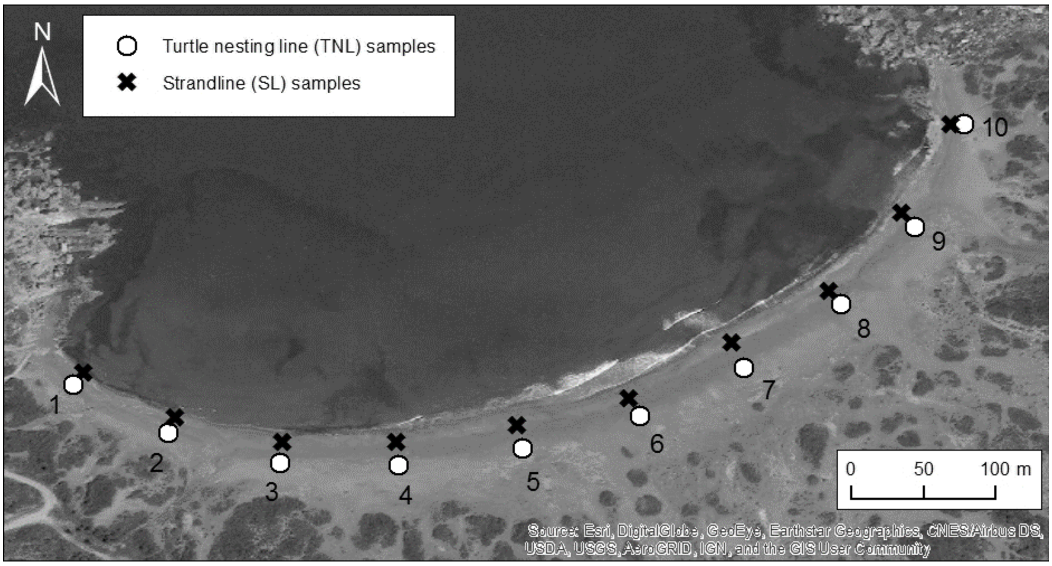

Figure S1. Experimental design of beach sediment sampling: 10 paired samples taken along the turtle nesting line (○) and strandline (**x**), plotted using GPS locations of samples taken on beach 5. Samples positioned ~34m from beach ends and ~68m apart on the 680m long beach

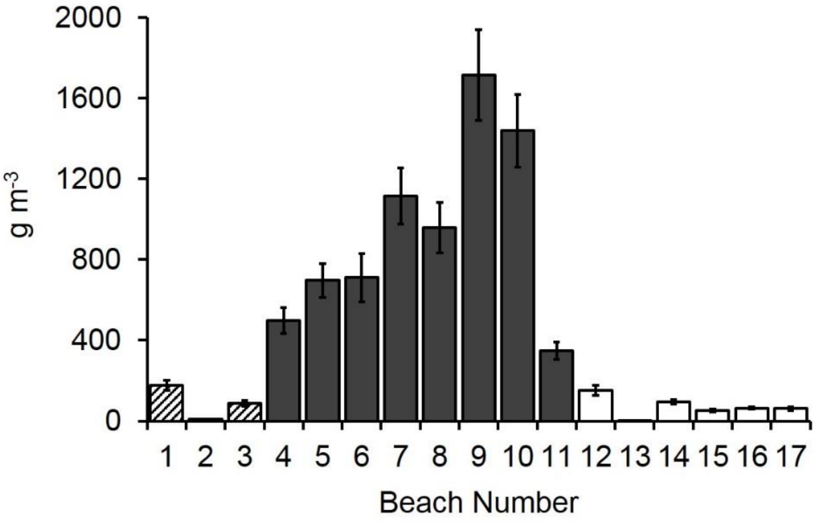

Figure S2. Mean microplastic in g m^{-3} within turtle nesting line (TNL) surface samples (0-2cm), across numbered sample beaches with fitted standard error bars. Striped= West (n=3, beach number 1-3), Grey = North (n=8, beach number 4-11), White = East (n=6, beach number 12-17).

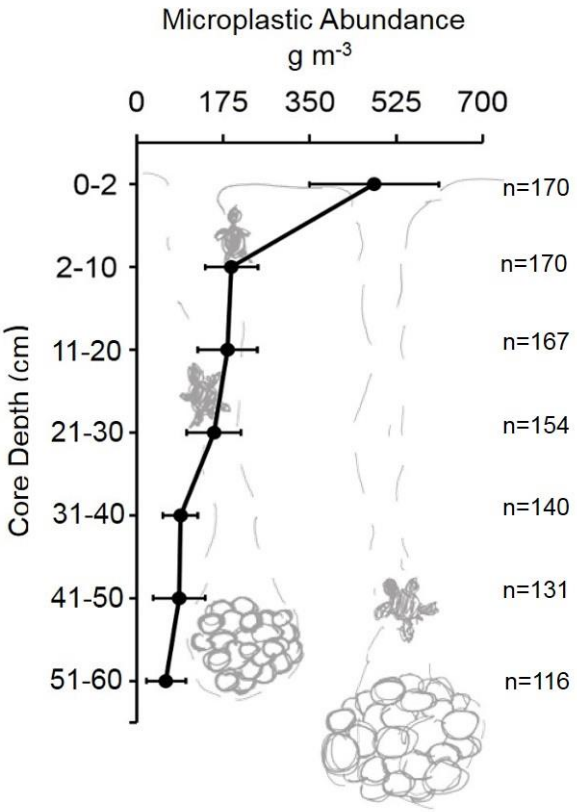

Figure S3. Grand mean (±S.E) of microplastic abundance in particles g^{-3} at different sand depths at turtle nesting areas (n=17 sites, n=170 sampling locations).

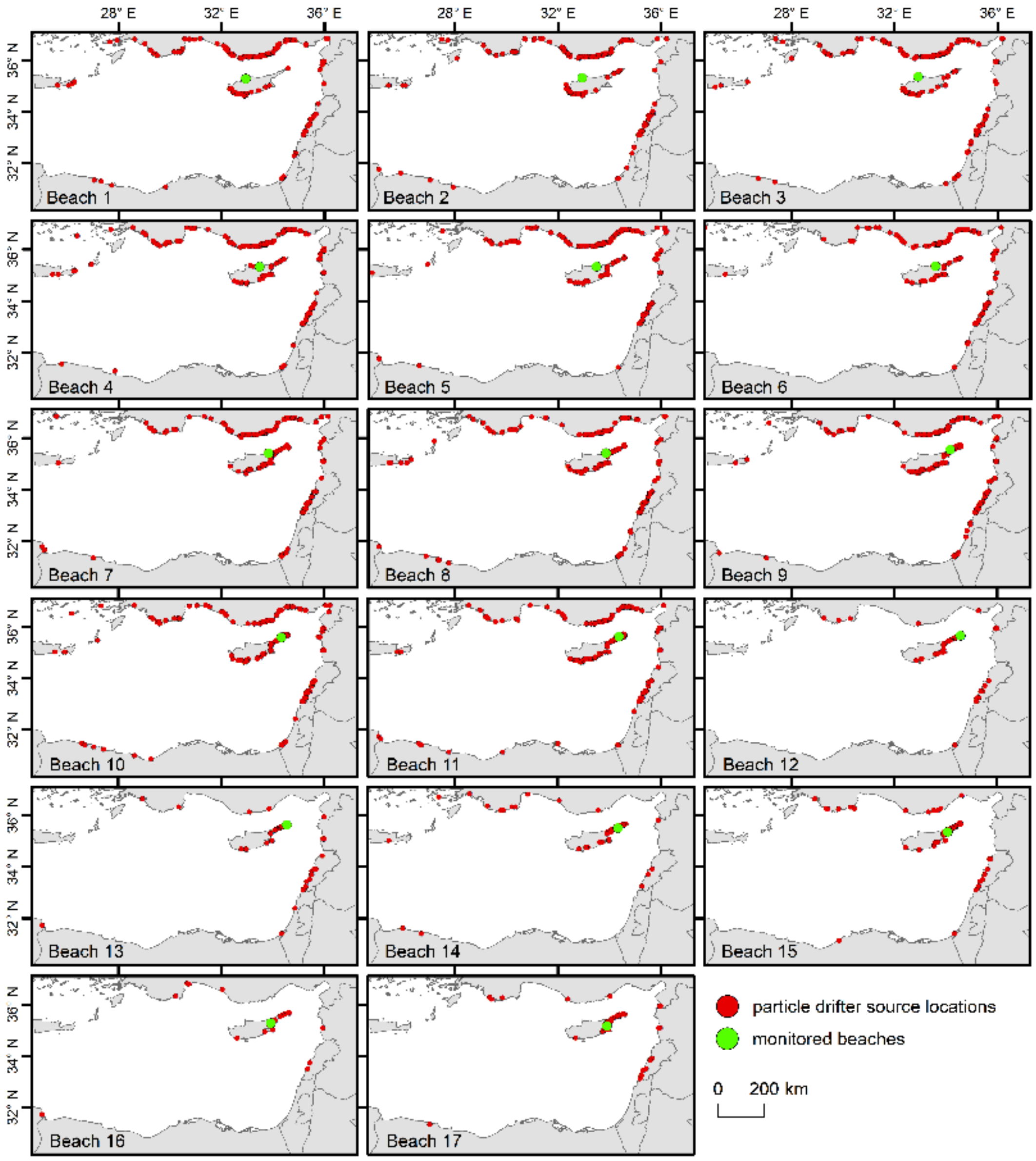

Figure S4. Maps the particle drifter source locations by monitored beach. These are locations where advected particles have become 'stuck' at their coastal 'sources'. Only locations that were within 5 km of the coast have been mapped

General Discussion

Overview

Marine turtles are potentially impacted by marine plastics by ingestion, entanglement, key habitat degradation and wider ecosystem effects. In the review **(Chapter 2)** I highlighted important research that urgently needs to be addressed to better understand the threat so that appropriate and effective mitigation policies can be developed. Throughout this thesis I have tackled and fulfilled a number of the recommended research priorities and knowledge gaps with in the field.

Entanglement

Entanglement is now recognised as occurring globally in marine turtle populations and is a documented cause of mortality. In **Chapter 3** by filling quantitative knowledge gaps on entanglement rates and populations implications, identifying challenges, research needs and priority actions we provide a baseline of knowledge for further action facing marine turtle entanglement (Duncan et al. 2017). It is clear that this issue of entanglement with plastic debris, such as ghost fishing gear, is both an under-reported and under-researched threat. It remains unclear whether this issue is more relative to animal welfare than substantive conservation concern of marine turtle populations. This cannot be answered however, until we improve capacity to report on incidence (Laist 1987, Vegter et al. 2014, Nelms et al. 2016). However the insights of our global experts highlights the importance of integrating a social science approaches. Surveying was a powerful tool on obtaining a tangible feel of the suggestive scale of the global issues, such as marine entanglement, where empirical data is lacking (Martin et al. 2012).

Macroplastic ingestion

Macroplastic ingestion (>5mm) is a widely recognised occurrence in all species of marine turtles however, the true mechanistic reason why this occurs has been under discussion (Nelms et al. 2016, Vélez-Rubio et al. 2018).The inclusion of detailed records of colour and shape in the plastic classification ingestion protocol in **Chapter 4** has allowed me to explore the concept of selectivity in ingestion to a higher level of detail than previously (Schuyler et al. 2014, Fukuoka et al. 2016). For example, green turtles in Northern Cyprus displayed strong diet-related ingestion towards plastic debris that resembles seagrass by texture, colour and shape. This is likely to be true in the other species, with their own individually specialised dietary niche demanding further investigation (Bjorndal 1997). Therefore, in the future it will be important for the research field, public awareness, media and policy for each species to be treated separately. The diversity of foraging ecologies are going to largely impact on the plastic debris ingested and therefore influence the vulnerability of each species (Clukey et al. 2017). The integration of detailed, established knowledge of feeding ecology and developmental biology will further our understanding of the physiological and mechanistic reasons behind the ingestion of debris present in the environment by marine turtles.

Microplastic ingestion

In **Chapter 5,** I developed a method for the quantification of microplastics (<5mm) in marine turtle gut content, adapting previous isolation methods used for plankton (Cole et al. 2013, 2014). This allowed the identification and isolation of a suite of synthetic particles in gut content residue samples, providing evidence of ingestion of synthetic debris at the microscopic size class. Unknown ingestion pathways are now evident and require further investigation. To answer this, a holistic approach will need to be adopted with sampling of all aspects of the environment and targeting specific dietary items; to aid in exploring the microplastic burden and the potential match to those ingested by marine turtles.

When considering impact, the question remains as to how much these truly microscopic plastic particles will be impacting on individuals. The size of them means that they will pass through the gut with relative ease (the possible exception being very small post-hatchlings) and therefore their presence does not lead to blockage or obstruction which is frequently reported in association with macroplastic ingestion (Ryan et al. 2016, Clukey et al. 2017). However the presence of microplastic particles in gut content does raise concerns regarding the accumulation of contaminants. It is widely thought that these particles can accumulate heavy metals, POPs and PCBs from the marine environment, in addition to the chemicals incorporated during production (such as plasticizers) that can potentially leach into biological tissue upon ingestion (Velzeboer et al. 2014, Nelms et al. 2016).

Key Habitats

In **Chapter 6** I have also explored the potential that plastic pollution could impact marine turtles not just through direct interaction with them but with their key habitats which they so heavily rely on; for example nesting beaches. The sampling protocol developed in this thesis not only captures data across the beach surface but also down to turtle nesting depth. To gain a more comprehensive viewpoint on plastic concentrations on nesting beaches, in the form of 3D sampling to investigate subsurface plastic densities, microplastics were identified down to turtle nesting depth of both loggerhead and green turtles in Northern Cyprus. If sediments for incubating eggs display extremely high plastic burdens incubation sex ratios and hatching success could be affected by changes to the nest microclimate and chemical contamination (Carson et al. 2011). Furthermore the integration of oceanographic modelling techniques allowed hindcasting of how key nesting beaches are likely to be impacted and potential source locations of the plastic debris (van Sebille et al. 2012, 2015).

Future Directions for research

It is clear that marine turtles are impacted and will continue to be impacted by plastic debris through diverse and widespread pathways. Given the increasing extent, scale and variability of both macro and microplastic pollution in the marine environment there is still much more to do to improve the knowledge of relative risk. Further research into specific species, populations and life stages will aid in building an understanding of the likelihood of exposure and consequences of ingestion and therefore overall risk. Finally to aid in building a holistic view of the impact of plastic pollution on marine turtles, assessment will need to be carried out in all key habitats, beyond nesting beaches; for example in foraging grounds and oceanic fronts. Protocol development will be key here for difficult sampling of waterborne plastic pollution.

Due to the increased public interest and exponential growth of research into the threat of plastic pollution there is an urgent need for standardisation of protocols for sampling and reporting on all aspects of the field to allow for comparable results where currently there is a lack of consistency. Furthermore, developing methods to sample from live turtles (such as faecal and lavage techniques) will assist in greater understanding of plastic burdens and diminishing the reliance on stranded animals for information on the scale of this issue. This will be especially important when considering targeted efforts to address geographic, species and life stage knowledge gaps, in addition to the development of body condition indices and ultimately culminating in a global database; only then can true population scale impacts become apparent (Nelms et al. 2016).

One of the areas that requires close attention is the difficulty in assessing and monitoring microplastics, and analytical chemistry to identify polymer type (Silva et al. 2018). Isolation of synthetic particles from marine turtle gut content requires further optimisation in terms of enzymatic digestion, to include elements such as sediment, chitin and plant based materials. In addition, advances in polymer identification of isolated particles will require collaborative work with the fields of chemistry and physics to gain precise results. Once levels of plastic contamination can be accurately assessed then pathological and toxicology links can be assessed.

Conclusion

In conclusion, marine turtles are impacted by plastic pollution in a myriad of ways; many of these urgently need more knowledge to assess the full risk. The exponential growth in this research area needs to be standardised and comparable to aid in a global understanding of potential impacts. This thesis forms the most detailed and comprehensive investigation to date on the impacts of this pollutant on the taxon of marine turtles; contributing to knowledge into macro and microplastic ingestion, entanglement and key habitats through method development and integration of marine turtle feeding ecology and developmental biology.

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