The cryptic and transboundary nature of ghost gear in the Maldivian Archipelago

Martin Richard Stelfox
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<th>Description</th>
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<tbody>
<tr>
<td>ALDFG</td>
<td>Abandoned, lost or discarded fishing gear.</td>
</tr>
<tr>
<td>CITES</td>
<td>Convention on International Trade in Endangered Species.</td>
</tr>
<tr>
<td>C-CAMP</td>
<td>Centre for Cellular and Molecular Platforms</td>
</tr>
<tr>
<td>CMP</td>
<td>Conservation and Management Plan</td>
</tr>
<tr>
<td>COFI</td>
<td>Committee on Fisheries</td>
</tr>
<tr>
<td>CYPAus</td>
<td>Cape York Australia</td>
</tr>
<tr>
<td>DAPC</td>
<td>Discriminate Analyses of Principal Components</td>
</tr>
<tr>
<td>dFADS</td>
<td>Drifting Fish Aggregating Devices</td>
</tr>
<tr>
<td>DVM</td>
<td>Devi River Mouth</td>
</tr>
<tr>
<td>EEZ</td>
<td>Exclusive Economic Zone</td>
</tr>
<tr>
<td>ESO</td>
<td>Environmental Society of Oman</td>
</tr>
<tr>
<td>EPA</td>
<td>Environmental Protection Agency</td>
</tr>
<tr>
<td>FAO</td>
<td>Food and Agriculture Organisation</td>
</tr>
<tr>
<td>FB</td>
<td>Flinders Beach</td>
</tr>
<tr>
<td>GGGI</td>
<td>Global Ghost Gear Initiative</td>
</tr>
<tr>
<td>GN</td>
<td>Gahirmatha</td>
</tr>
<tr>
<td>GPS</td>
<td>Global Positioning System</td>
</tr>
<tr>
<td>IMO</td>
<td>International Maritime Organisation</td>
</tr>
<tr>
<td>INDELS</td>
<td>Insertions and Deletions</td>
</tr>
<tr>
<td>IOSEA</td>
<td>Indian Ocean South East Asian</td>
</tr>
<tr>
<td>IOTC</td>
<td>Indian Ocean Tuna Commission</td>
</tr>
<tr>
<td>IUCN</td>
<td>International Union for Conservation of Nature</td>
</tr>
<tr>
<td>IUU</td>
<td>Illegal, Unreported and Unregulated</td>
</tr>
<tr>
<td>MAD</td>
<td>Madras</td>
</tr>
<tr>
<td>MG</td>
<td>McCluer Group</td>
</tr>
<tr>
<td>ML</td>
<td>Monofilament Line</td>
</tr>
<tr>
<td>MRS</td>
<td>Marine Research Centre of the Maldives</td>
</tr>
<tr>
<td>mtDNA</td>
<td>Mitochondrial Deoxyribonucleic Acid</td>
</tr>
<tr>
<td>mv</td>
<td>Medium Vectors</td>
</tr>
<tr>
<td>N</td>
<td>Nets</td>
</tr>
<tr>
<td>NGO</td>
<td>Non Governmental Organisation</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
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<tr>
<td>--------------</td>
<td>-------------</td>
</tr>
<tr>
<td>NL</td>
<td>Net Line combination</td>
</tr>
<tr>
<td>NOAA</td>
<td>National Oceanic and Atmospheric Administration</td>
</tr>
<tr>
<td>NTAus</td>
<td>Northern Australia</td>
</tr>
<tr>
<td>ORP</td>
<td>Olive Ridley Project</td>
</tr>
<tr>
<td>OSCUR</td>
<td>Ocean Surface Current Simulator</td>
</tr>
<tr>
<td>OSCAR</td>
<td>Ocean Surface Current Analysis Real-time</td>
</tr>
<tr>
<td>PCR</td>
<td>Polymerase Chain Reaction</td>
</tr>
<tr>
<td>PERMANOVA</td>
<td>Permutational Multivariate Analyses of Variance</td>
</tr>
<tr>
<td>RPT</td>
<td>Rope attached to Pots and Traps</td>
</tr>
<tr>
<td>RU</td>
<td>Rushikulya</td>
</tr>
<tr>
<td>SL</td>
<td>Sri Lanka</td>
</tr>
<tr>
<td>TI</td>
<td>Tiwi Island</td>
</tr>
<tr>
<td>UR</td>
<td>Unknown Rope</td>
</tr>
<tr>
<td>WWF</td>
<td>World Wildlife Fund for Nature</td>
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</table>
Abstract
Abandoned, lost or discarded fishing gear (ALDFG), more commonly referred to as ghost gear, is a global issue that impacts many marine organisms worldwide. In the Maldivian archipelago a large number of olive ridley sea turtles (*Lepidochelys olivacea*) are found entangled in these nets (more commonly referred to as ghost nets) each year. However, the origin of these nets or turtles are unknown considering fishing with the use of nets is restricted to the bait fisheries within the exclusive economic zone of the Maldives. Therefore, ghost gear has a transboundary and cryptic nature, making it difficult to assess the environmental impact and origin of the gear. This thesis aimed to develop new tools and techniques which could be utilised to examine these unknowns.

I revealed in a literature review (Chapter 1) that research in ghost gear entanglements amongst marine megafauna are predominantly focussed in the Atlantic and Pacific Ocean. However, Indian, Arctic and southern Oceans are far less studied. Additionally, the majority of strategies to tackle ghost gear were centred around curative measures, such as ghost gear retrieval. I advise that future solutions, best practices and research should favour preventative rather than curative methods in ghost gear management and research.

Statistical classifies (Chapter 2) were built in R to predict the probability of a net entangling a turtle. It was highlighted that nets with larger mesh sizes and the absence of floats were major gear characteristics that increased the likelihood of turtle entanglement. In addition, the time of year was an important variable with a higher chance of turtle entanglements in nets found during the northeast monsoon (November – April). Unfortunately, grouping of the nets by fisheries was not possible, beyond a broad classification. This was likely a result of the wide variety of nets used in the region. However, gill and trawl nets were recognised as high-risk fisheries. Regardless of the difficulties of assigning an origin of the nets, I was able to estimate the scale of the problem. Between 3,400 and 12,200 turtles could have become entangled in ghost nets over the 51-month study period, meaning this region has the highest turtle entanglement rate in ghost nets worldwide (0.17).

Nesting and sightings of free-swimming individuals are rare and therefore the majority of entangled turtles do not originate from the Maldives. To discover the source population of these entangled olive ridleys we utilised a mixed stock analyses of mtDNA from samples of turtles entangled in nets in the Maldives (n = 38) and compared them to nesting stocks from published literature (Sri Lanka, east India and northern Australia). We were able to fill in data gaps in phylogenetics by including samples from previously undescribed nesting populations, such as those in Oman and improved resolution by including longer sequences from east India in our analyses (Chapter 3). Results suggest that the majority of entangled olive ridleys originate from east Indian (73%) and Sri Lankan (23%) genetic
stocks when no population estimates were included in model design. This meant we could estimate the impact of ghost nets on these populations. Recorded ghost net entanglements may impact yearly recruitment of east Indian populations by 0.48% however a staggering 41% of the Sri Lankan population are thought to be negatively affected by the drifting nets.

I then attempted to age ghost gear found drifting in the Maldives, and provided additional evidence to locate possible sites of origin. Percentage cover of biofouling communities and capitulum length of the goose barnacle (*Lepas anatifera*) provided the most robust metrics to estimate minimum drift times ([Chapter 4](#)). Lagrangian simulations (forced by Ocean Surface Current Analyses Realtime OSCAR) could then be utilised to backtrack drifting ghost gear to a putative origin. This analysis highlighted that the origin of these nets overlapped with purse seine (predominantly from Spain and France) and gill net fisheries operating in the area. Moreover, the models show that some of the nets originate close to the Indian and Sri Lanka shorelines, suggesting that small scale artisanal fisheries may provide additional high risk, contributing to ghost nets drifting into the Maldives and entangling turtles.

In summary it is hoped that this thesis advances our knowledge on ghost gear significantly. Moreover, this thesis provides the information and tools necessary for the Olive Ridley Project (a British registered charity, tackling this issue face on), along with other stakeholders (government and non-government) in order to better manage resources and combat the ghost gear issue within the Indian Ocean.
Preface

The work, including conceptual design of experiments, data collection and writing within this thesis, has been solely authored by the doctoral candidate with guidance from the supervisory team. Guidance for the statistical analysis and R coding was provided by Dr. Mark Bulling and Dr. Alfred Burian. Any images used that were not taken by the candidate have been credited appropriately with their permission. Where chapters have been published the doctoral candidate has been the lead author, preparing the manuscript, with only guidance from co-authors. The work contain within this thesis has been disseminated through a number of channels, listed here:

Publications


Conferences


**Stelfox, M.**, Bulling, M. and Sweet, M. (2016). Statistical approaches to understanding the source and impact of ghost gear entanglement of *Lepidochelys olivacea* in the Maldivian Archipelago; Environmental Sustainability Research Centre annual conference, University of Derby, May.


Public outreach and press

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Acknowledgements

After finishing my bachelor’s degree over 15 years ago the thought of studying towards a PhD crossed my mind on several occasions. However, I questioned my ability to take on such a monumental undertaking and always put it off. Therefore, I would like to extend my sincere gratitude to my good friend and director of studies, Dr. Michael Sweet, for giving me this opportunity and believing in my ability to complete this study. Your ‘can do’ attitude and round the clock support made me believe in myself and helped me achieve what I thought was almost impossible. To that note I would also like to thank my supervisors Dr. Nel Beaumont and Dr. Maren Huck for steering me in the right direction early on in my PhD and providing encouragement and support to believe I could do it.

I knew from the start that the greatest hurdle I would have to face would be the statistics as this was never a strong point in my academic career. Thankfully I now feel that I can take on almost any statistical challenge and possible may have found a new passion. I owe my new love for statistics to Dr. Mark Bulling and Dr. Alfred Burian, your patience, commitments and passion helped me see this subject in a different light. Most of all, you helped me get through those moments when the only thing left to do was to bang my head against a wall in frustration at coding errors. I really look forward to working with you both and I’m excited to collaborate in the future.

This thesis would not have been possible without the support and encouragement from so many different partners. They understand the seriousness of ghost gear and without question provided me with all the necessary tools to complete my experiments and find solutions to the issue. First and foremost, I would like to thank the staff and volunteers at the Olive Ridley Project (ORP) for all their support throughout my studies. Since setting up the project in 2013 I have seen the team grow into the passionate and unstoppable force they are today. I would particularly like to thank Jannicke Hallum for always picking up loose ends as I strive to juggle work and PhD life. To that note I would also like to thank Mark Macdonald, Matthew Shoulders and Mike Sweet for keeping the charity ticking over whilst the PhD was getting completed. I would like to say a special thank you to Dr. Jillian Hudgins for your continued passion and presenting some of my research at conferences when I was not able to attend! Thank you to Jenni Choma, Sonia Valladares, Claire Nerissa, Claire Petros, Kylie Merritt, Deborah Burn, Tess Moriarty, Ghazi al Farsi, Juma Al Humaidi, Juma Al Araimi, Priyanka Swamy, Christophe Lett, Geraldine Reid and Josie Chandler for helping with collecting tissue samples and/or setting up my crazy experimental designs without hesitation. Without you guys this research would not have been possible.
I would also like to give special thanks to Andrew Willson of Five Oceans Environmental Services LLC for survey design and tissue collection in Oman and to Brendan Godley for his assistance with samples from Oman. Thank you to Amy Frey and Peter Dutton from the National Oceanic and Atmospheric Administration (NOAA) for helping me follow an international template for haplotype names that will hopefully form the new reference when naming olive ridley haplotypes in future publications.

Thank you to the team at Surescreen Scientifics, particularly Troy Whyte and Arthur Green for allowing me to use the SEM and putting up with my relentless questions! Thank you to Gareware Wall Ropes Ltd. for supplying me with nets and Coco collection Bodu Hithi, North Male, Six Senses, Laamu and Gilli Lankan Fushi, North Male in the Maldives, for allowing my experiments to take place in their lagoons.

Collaboration between different organisations was a major component to my research and therefore I would like to thank the Environmental Society of Oman (ESO), University of Exeter, Environmental Protection Agency (EPA) of the Maldives, Kelonia and the University of Derby.

The person I need to thank the most is my beautiful partner Jennifer. Your support has been selfless and inspiring. Doing a PhD whilst working at the same time is challenging and has led to many late and often sleepless nights. You stood by me the whole time and encouraged me to believe in myself which helped me get to the finish line. You are my rock and I cannot thank you enough, now it is my time to be your rock. I love you always and forever.

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General Introduction

Abandoned, lost or discarded fishing gear (ALDFG) more frequently referred to as ghost gear is recognised as a global concern within marine systems (Edward et al., 2020, Sweet et al., 2019, Goodman et al., 2019, Moschino et al., 2019, Parton et al., 2019). Despite this concern only a handful of studies have attempted to quantify the issue and assess its impact at the population level (Jensen et al., 2013, Wilcox et al., 2015). One of the major challenges when trying to quantitively estimate species mortality from ghost gear entanglement or ingestion is its cryptic and transboundary nature (Laist and Wray, 1995, Wilcox et al., 2015). Ghost gear is often unmarked and intercepted at the end of its life cycle, and therefore very little is known about how long the gear may have been drifting for, where it may have come from or its interactions along the way. Moreover, traditional materials such as cotton, hemp and coconut fibres, that once dominated the construction of fishing nets have been replaced with environmentally resistant polymers that do not biodegrade and stay in the environment for long periods of time (Kim et al., 2020). Plastic buoyant fishing nets are able to travel with ocean surface currents for long periods of time, insidiously killing marine organisms through ingestion (Reinert et al., 2017, Link et al., 2019) or entanglement (Stelfox et al., 2015, Wilcox et al., 2015, Duncan et al., 2017) in a process called ghost fishing which is often depicted in the media through shocking images of entangled turtles, whales and dolphins. Moreover, ghost gear can sink to the ocean floor and smother benthic communities (Watters et al., 2015, Goodman et al., 2020) and sensitive habitats such as coral reefs (Donohue et al., 2001, Ballesteros et al., 2018) further adding to the complexity of trying to quantitively and qualitatively assess the impact to ecosystems as a whole. Researchers and conservationists need to better understand the issues surrounding ghost gear and ghost fishing to ensure that existing conservation or management strategies are not being undermined.

Maldivian archipelago and ghost gear

The Maldivian archipelago consists of around 1190 islands divided into 26 atolls covering almost 1 million km² within its exclusive economic zone (Adam, 2006). The archipelago is subject to two major monsoons, the north east (NE) monsoon where surface currents broadly travel east to west between November and April and the south west (SW) monsoon where currents travel from west to east between May and October (Shankar et al., 2002). There are two major industries in the Maldives; tourism and fishing (Adam et al., 2006). The fishing industry is largely centred around the pole and line and handline targeting skipjack (Katsuwonus pelamis) and yellowfin tuna (Thunnus albacares; Adam et al., 2014). At the core of the tuna fishery is the bait fishery targeting small pelagic and reef associated species such as silver sprat (Spratelloides gracilllis), blue sprat (Spratelloides delicatulus), anchovy
(Encrasicholina heteroloba) and fusiliers (Caesionidae sp.; Jauharee et al., 2015). Typically pole and line fishers use night lights to attract bait to the surface where they are then scooped up by a small meshed hand net (the only type of nets allowed to be used in the Maldives). In addition to the pole and line fishery, the Maldives have anchored fish aggregating devices (aFADs) deployed in deep water within its EEZ. Fishers using aFADs are able to capitalise on the phenomenon of pelagic species congregating under floating flotsam or debris by mimicking its effect under manmade structures (Adam et al., 2019). Interestingly, despite the use of nets being prohibited in the Maldives, reports of ghost gear, particularly ghost nets are common (Anderson et al., 2004, Stelfox et al., 2014). Therefore, it is reasonable to assume that the majority of ghost nets found originate from outside the Maldivian archipelago from neighbouring countries, brought in by the changing monsoons or from illegal fishing activity within the EEZ. Moreover, these nets often bring with them entangled sea turtles, predominantly olive ridley sea turtles (Lepidochelys olivacea). This points to further evidence that nets originate from outside the Maldives since this species only nest in the area on very rare occasions and no foraging habitats have been identified in the region for this species so far.

**Background information on the work associated with the following chapters**

The impact of ghost gear in the Maldives was first documented in 2009 as a technical report for the Indian Ocean Tuna Commission (IOTC) (Anderson et al., 2009). In this report the authors compiled all historical records of entangled olive ridley turtles up to 2003. A total of 25 turtles were recorded with a peak during the north east (NE) monsoon. Speculation as to the origin of the entangled sea turtles were attributed to the east coast of India, with suggestions of nets originating from Indian and Sri Lankan fisheries. However, these assumptions were not evidence based and low reporting made it difficult to ascertain robust trends.

In response to the number of entangled sea turtles documented floating in the Maldives I decided to develop a project to better understand the extent of the issue. With the help of fellow biologists David Balson and Dr. Jillian Hudgins I began recording all ghost net and turtle entanglement encounters across the archipelago. I then set up a closed Facebook group for all biologists working in the Maldivian archipelago with a specific aim to facilitate recovery of ghost nets, rescue entangled turtles and collect data on ghost net findings. Shortly after, the project was named the ‘Olive Ridley Project’ (ORP) after the predominant species found entangled. It later became a registered UK charity in March 2016 (charity reg. #1165905) and has since broadened its objectives to protect sea turtles and their habitats across the wider Indian Ocean.
The ORP now has one of the largest ALDFG and turtle entanglement databases in the northern Indian Ocean and one of the world's largest capture-mark-recapture projects using photo-ID with over 20,000 sightings consisting of 3200 individual hawksbill turtles (Eretmochelys imbricata) and 660 green turtles (Chelonia mydas) recorded in the Maldives between 2013-2018. The project has the first and only veterinarian run rehabilitation centre in the Maldives and has developed turtle research and ghost net projects across four countries: Oman, Maldives, Kenya and Pakistan.

In the early project implementation, I collaborated with the International Union for Conservation of Nature (IUCN) and the Marine Research Centre of the Maldives (MRC) to develop a standardised database for recording ghost net characteristics. The net toolkit developed by the World Wildlife Fund (WWF) in northern Australia (White et al., 2004) was used as a reference point for developing a protocol specific to the region. After feedback from numerous participating marine biologists and several focus workshops to develop the protocol, ORP later modified the method to ensure all relevant data fields were present and removed. These included those that were either: i) difficult to record in the field or ii) rarely recorded by participating citizen scientists; https://oliveridleyproject.org/report-a-ghost-net (Appendix 1)

Since the early report by Anderson et al. (2009), ORP has recorded a further 1440 ghost net fragments entangling 582 olive ridley, 33 hawksbill, 13 green turtles and 1 Leatherback (Dermochelys coriacea) between July 2013 – June 2019. Entangled olive ridley turtles measured between 12 cm – 75 cm curved carapace length and almost half of individuals were classed as juveniles or sub-adults (n = 274), i.e. 60 cm or smaller (Appendix 2). Ghost nets were reported in various sizes, ranging between 6 – 9700 mm stretched mesh size and 0.2 – 15 mm twine diameter. The expanse of the Maldivian archipelago (298 square kilometres) means that it is highly likely that many events go undetected therefore these reports are likely an underrepresentation of the true number of ghost nets drifting into the Maldives.

In this study “The Transboundary and Cryptic Nature of Ghost Gear in the Maldivian Archipelago” I explore the impact of ghost gear in the Maldives and its perceived threat to olive ridley sea turtles (Lepidochelys olivacea). In addition, I assed drift trajectories and use novel statistical tools to uncover the cryptic and transboundary nature of ghost gear and identify responsible fisheries.

In Chapter 1 “A review of ghost gear entanglement amongst marine mammals, reptiles and elasmobranchs” I review the available literature on ghost gear and ghost fishing and its impact to marine megafauna globally to identify current data gaps in ghost gear research to help direct research goals in this study. I also assess the different management strategies implemented around the world to
help tackle the issue.

In Chapter 2 “Untangling the origin of ghost gear within the Maldivian archipelago and its impact on olive ridley (Lepidochelys olivacea) populations” I develop statistical tools exploring key drivers in fishing net design and seasonality that increases the likelihood of olive ridley entanglements in the Maldives. Moreover, a novel clustering algorithm was used to broadly categorise ghost nets collected in the Maldives to responsible fisheries. Through these methods I was able to estimate the number of olive ridleys entangled by ghost nets over a 51-month period prior to being found entangled in the Maldives. I call for improved ghost gear data collection and discuss management recommendations in the region.

In Chapter 3 “Minimum drift times infer trajectories of ghost nets found in the Maldives” I exploit the phenomena of bioaccumulation to determine how long ghost nets may have been exposed to sea water. From this I was able to estimate minimum drift times and use lagrangian simulations to explore drift trajectories of ghost nets found in the Maldives. I uncovered the transboundary nature of ghost nets through different maritime political borders and improve uncover the cryptic movements of ghost gear found in the Maldives. I was then able to overlap areas of operation of different fisheries reporting to the Indian Ocean Tuna Commission (IOTC) with drifting pathways to detect probable fisheries responsible for ghost gear drifting into the Maldives.

In Chapter 4 “Tracing the origin of olive ridley turtles entangled in ghost nets in the Maldives: A phylogeographic assessment of populations at risk” I assess mitochondrial DNA (mtDNA) to analyse the phylogeographic relationship of nesting olive ridleys in the Indian Ocean to determine an origin of entangled olive ridleys found in the Maldives. This improved genetic resolution helped identify entangled turtles to a population and for the first time quantitively estimate this previously undocumented threat to each impacted population.

In Chapter 5 “General discussion of thesis findings and future directions” I summarise the key findings of my thesis chapters and highlight the application of my research in the field. I highlight how this research can help to guide future management decisions of the ghost gear issue by the Olive Ridley Project. Additionally, I pull on my experience working on a successful citizen science project to propose how data collection by the Olive Ridley Project may be improved to maximise volunteer data contribution. I finally provide evidence-based direction for future research and suggest a call to action to help mitigate the impact of ghost gear by fisheries in the region, particularly relating to fish aggregating devices (FADs).
This thesis is particularly timely given the recent coverage on plastics in the media and increasing interest within the scientific community. It also fits nicely within the context of the Global Ghost Gear Initiative (GGGI), a cross sectoral alliance of NGO, IGO, private sector and governments working to solve the ghost gear issue globally. It is hoped that the research in this thesis will contribute to our lack of understanding of ghost gear and ghost fishing and pave the way for experimental replication in other regions of the globe to initiate ghost gear recording and analyses.
References


Sensitivity: Internal


*Lepidochelys olivacea* in ghost nets in the central Indian Ocean: *BOBLME-2015-Ecology* 14,
pp.1–23.


the sources and effects of abandoned, lost, and discarded fishing gear on marine turtles in

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Chapter 1: A review of ghost gear entanglement amongst marine mammals, reptiles and elasmobranchs

1.1 Abstract

This chapter focuses on the effect that ghost gear entanglement has on marine megafauna, namely mammals, reptiles and elasmobranchs. A total of 76 publications and other sources of grey literature were assessed, and these highlighted that over 5,400 individuals from 40 different species were recorded as entangled in, or associated with, ghost gear. Interestingly, there appeared to be a deficit of research in the Indian, Southern, and Arctic Oceans; and so, this study recommends that future research focus efforts on these areas, hence why the remainder of my thesis explored ghost gear and entanglement within the Indian Ocean or more specifically the Maldivian archipelago. Furthermore, but not the focus of my thesis, this chapter indicated that studies assessing the effects of ghost gear on elasmobranchs, manatees, and dugongs should also be prioritised, as these groups were underrepresented in the current literature. The development of regional databases, capable of recording entanglement incidences following a minimum global set of criteria, was identified as a logical step in order to analyse the effect that ghost gear has on megafauna populations worldwide and again part of this thesis utilised exactly this.

1.2 Introduction

Though fishing gear has been lost since fishing began, historically such gear was made from natural materials that would have decomposed quickly and created a relatively small threat to marine wildlife. However, in recent years advances in technology and improvements in gear designs have forced fishers to switch to gear made from synthetic materials, namely plastics (e.g. Macfadyen et al., 2009). Synthetic fishing materials such as nylon, polyethylene, and polypropylene take a long time to degrade and tend to accumulate in the marine environment for long periods of time (Barnes et al., 2009). This promotes a phenomenon called ‘ghost fishing’, whereby lost or discarded gear continue to catch an abundance of wildlife from a range of taxa (Stelfox et al., 2015, Wilcox et al., 2015, Masompour et al., 2018). The actual amount of abandoned, lost, or otherwise discarded fishing gear (often shortened to ALDFG) is extremely difficult to quantify. However, it has been estimated that each year, upwards of 640,000 tons of gear is lost globally, meaning that ALDFG accounts for over 10% of the total marine debris floating in our oceans (Macfadyen et al., 2009). Given that survey effort for ALDFG is often poor or sporadic in many areas around the world, this 10% is therefore likely to be a gross underestimate.
of the true amount. As early as the 1980s, the Food and Agriculture Organisation (FAO) recognised ALDFG as a global problem. ALDFG also fits under the mandate of the International Maritime Organisation (IMO), which heads the International Convention for the Prevention of Pollution from Ships (MARPOL Annex V). Furthermore, the issue has been raised at numerous United Nation general assemblies (Macfadyen et al., 2009). Although ALDFG is clearly a global concern affecting many species, it is important to pay particular attention to those species that are sensitive to anthropogenic stress (i.e. those with low fecundity or increased age at sexual maturity). This chapter focusses on how entanglement in ALDFG impacts marine megafauna, such as mammals, reptiles and elasmobranchs.

Numbers were pulled from all traceable literature dating back to the last comprehensive review conducted by Laist (1997). One other major aim of this chapter was to identify the different gear types that contribute to ghost fishing and give recommendations on how to manage and mitigate the issue.

1.2.1 Supply and demand - A brief look at global fishing pressure

Fishing pressure has increased dramatically worldwide since the 1970s (Anticamara et al., 2011). It is thought that a growing global human population, combined with higher incomes and an insatiable appetite for seafood around the world, were likely factors contributing to this observable increase (Swartz et al., 2010). To date, 28.8% of global fish stocks are thought to be over-exploited with a further 61.3% fully fished out with no room for further expansion of catch (FAO, 2014). Advances in technology have created powerful mechanised fishing vessels that are capable of fishing on a global scale and at increasing depths. An estimated 4.72 million fishing vessels were legally operating in 2012, of which 57% were engine powered (FAO, 2014). Although these figures are staggering, they do not take into account illegal, unreported, and unregulated (IUU) fishing, or those countries that failed to submit data to the FAO database. Therefore, the actual fishing pressure in the world's oceans is likely to be significantly higher. Due to increasing demand, higher competition, and reduced fish stocks, fishers around the world, both small local artisan fishers and commercial operations, have had to change their fishing techniques and increase their effort by switching to increasingly more durable and longer lasting materials as a response (Carr and Harris, 1997). For example, artisan fishers in many states of India were first introduced to cheaper nylon monofilament nets as late as 1990, and by the early 2000s monofilament nets had almost completely replaced multifilament nylon nets in small and large-scale gill net fisheries. Fishers in other parts of South East Asia are now increasingly favouring thinner nets, referred to as 'superfine nets. Unfortunately, the thinness of the twine means that the nets break easily and, unlike stronger multifilament nets, become difficult to repair once damaged (Rao, 2010). Similarly, by the late 1980s traditional fishers in parts of Queensland, Australia, started to favour fishing with pots made from multifilament polyethylene trawl mesh over the less durable pots.
constructed of wire mesh and steel that would corrode quickly (Campbell and Sumpton, 2009). This change in gear design was most likely driven by the cost of the new nets and pots, as they are cheaper to purchase and easy to replace if, or when, they are damaged.

1.2.2 When does fishing gear become ALDFG?

Fishing gear becomes ALDFG when the fisher loses all operational control of the equipment (Smolowitz, 1978). A switch from the natural or biodegradable material that was traditionally used for centuries to synthetic fishing gear (as described above) has led to a rise in the amount of ALDFG accumulating in the world's oceans. The causes of ALDFG include snagging on the bottom, storms or bad weather, operational damages, improper gear use, gear conflicts (conflicts between fisheries), high cost relating to proper disposal, lack of disposal facilities, lack of space on fishing craft, and logistical difficulties retrieving gear. For a summary of the multitude of reasons why fishing gear may be lost refer to Macfadyen et al. (2009) for more information.

1.2.3 What factors affect ghost fishing rates?

Ghost fishing is defined as the ability of fishing gear to continue to fish after all control of that gear is lost (Smolowitz, 1978). This definition, however, does not give specifics on how to identify mortality rates associated with ghost fishing. Matsuoka et al. (2005) suggested that the presence of lost fishing gear and the entry of organisms into that gear, for example, was not substantial enough evidence to prove that the gear was ‘ghost fishing’. Additionally, the identification of any dead animals must be conducted to species level in order to give reliable mortality rates of ghost fishing. The survival rate of animals that have escaped entanglement must also be considered; a problem that is more difficult to quantify.

Using a terrestrial example of entanglement and mortality, a study by Votier et al. (2011) argued that colonial seabirds released from entangling plastic would not survive without human intervention. They suggested that individuals that had escaped or were released from the gear would likely succumb to death as a direct result of the entanglement and should, therefore, also be considered in the mortality estimate. Mortality due to ghost fishing is therefore very difficult to quantify. Early studies suggested that the rate of ghost fishing may be influenced by numerous factors including: the abundance of fauna in any given area, the environmental conditions that the gear is exposed to at any given time (such as currents or storms), and the habitat type (Kaiser et al., 1996). Unsurprisingly, there was a general consensus in the literature that static ghost nets show a general decline in catch rates over time (e.g. Humborstad et al., 2003, MacMullen et al., 2003, Revill and Dunlin, 2003, Tschernij and Larsson, 2003). For example, in-water observations of lost gill nets in the relatively sheltered waters of the Baltic
Sea, suggested that catch efficiency rapidly deteriorates and then stabilises at around 20% of the original level after three months. After 27 months, the level of ghost fishing efficiency is reduced further to approximately 5–6% of the original level (Tschernij and Larsson, 2003). However, the catch efficiency of set nets will depend on the net structure and this can be affected by the presence or absence of obstacles such as wrecks or rocky bottoms versus smooth sandy bottoms or deep-water. A net has a greater chance of being snagged in situ on an obstacle, such as a rocky bottom or coral reef, which may tear the mesh creating larger holes for larger animals to become entangled. Conversely, if a net is set and lost on a shallow sandy bottom, it will continue to ghost fish until the weight of the catch reduces the vertical height of the net and it ends up as a pile on the sea floor with little to no fishing ability (Baeta et al., 2009).

Since the structure of fishing gear is an important factor when determining ghost fishing efficiency, it was suggested that traps and pots may be more prone to ghost fishing simply because they are made of longer-lasting, more rigid materials that maintain their optimal configuration over time (Adey et al., 2008). For example, early studies on the escape rate of Dungeness crabs (Metacarcinus magister) from pots in the Columbia River estuary report mortality rates of legal sized crabs to be 52% (Breen, 1987); however, similar studies of baited creels on Norwegian lobsters (Nephrops norvegicus) showed a much lower ghost fishing efficiency. This was attributed to a gear design that allowed other non-target species to escape and the unique ability of Norwegian lobsters to survive long periods after they are caught (Adey et al., 2008). Although such types of ALDFG pose little direct threat to marine megafauna, simply due to the size of the animals, the associated lines that connect the traps and pots to the surface could be a considerable entangling threat to these groups. Furthermore, the traps may also act as a potential food source increasing the likelihood of megafauna coming into contact with the traps, fragments of them, or the associated fishing lines. This highlights an indirect effect that some types of ALDFG may have on certain species or taxa. For example, lobster fishers on the Atlantic side of the Florida Keys recorded dolphins and turtles breaking gear as they foraged for lobsters caught inside the traps (Butler and Matthews, 2015); however, no data has been published highlighting if any indirect entanglements occurred due to this opportunistic feeding behaviour.

Considerable literature exists on the rate of ghost fishing for static ALDFG; however, little information is available for transient ALDFG that follow winds and geostrophic currents. Fragments of nets or drifting fish aggregating devices (dFADs) are extremely difficult to track or find, making prolonged studies very challenging. Most information available on this cryptic gear is from anecdotal records since the costs and logistics involved in a wide scale study are prohibitive. It is tempting to assume that the rate of ghost fishing for this type of ALDFG may follow the same influencing factors as for static
gear, but no conclusions can be drawn until more research is focused on this type of gear.

Ghost fishing rates are likely also biased towards survey effort (i.e. the more time put into the survey, the more animals are likely to be found entangled). For example, eight survey cruises at 11 breeding colonies of Californian sea lions (Zalophus californianus) between 1991 and 1995, recorded entanglement rates between 0 and 2.24% (Zavalagonzalez and Mellink, 1997). A similar survey of grey seals (Halichoerus grypus) in the UK reported entanglement rates of 3.1 - 4.9% (Sayer, 2015); however, survey effort was much greater in the latter study, with surveys averaging 226 days out of 365 between 2004 and 2013. Similarly, early data collected for entangled olive ridleys turtles (Lepidochelys olivacea) in the Maldives reported only 25 incidences of entanglements over eight years between 1999 and 2007 (Anderson et al., 2009); however, with an increase in recording efforts, this number rose to 163 in just two years (2013 - 2015) (Stelfox and Hudgins, 2015).

The above illustrates the importance of incorporating a unit of effort metric when describing the effect of ghost gear on any specific organism. Furthermore, the difficulty in getting to certain locations coupled with local weather conditions can make observing entangled animals and empty nets difficult (Boren et al., 2006). Therefore, it is very important to note and understand that any estimated entanglement rates in the literature represent only instantaneous measures of entanglement or mortality since not all entangled animals can be observed at any given time (Henderson, 1984, Matthews and Glazer, 2010).

1.2.4 The cyclical nature of ghost fishing and the role of biofouling

ALDFG that remains in water for long enough will eventually accumulate sessile organisms in a process referred to as ‘bio-fouling’. The time it takes to accumulate such biofouling greatly depends on environmental factors such as temperature, location, etc. (Bixler and Bhushan, 2012). It was suggested that biofouling could be one reason why ghost fishing efficiency decreases with time (as described above), as the net becomes more visible to animals (Revill and Dunlin, 2003). Visibility of nets likely has a major effect on ghost fishing efficiency. Monofilament nets have higher catch rates than multifilament nets and it is thought that the higher visibility of the multifilament nets is the main reason for this difference (Ayaz et al., 2006). On the other hand, floating ALDFG with significant biofouling may attract small animals looking for food and shelter, which in turn would attract larger predators (such as turtles, cetaceans, sharks, etc.). This could give rise to a continuous cycle of ghost fishing initially brought on by biofilm build-up (Carr, 1987). Biofouling can also assist researchers in aging the ALDFG. For example, analyses of size classes of common bivalve species on nets showed that shell length of Anomia sp. can be used to age ALDFG in the Bay of Biscay (Pawson, 2003). The amount of bio-fouling on any given net, along with the species found (which varies depending on geographic
location), could, therefore, be used as a tool to calculate approximate drifting times of ALDFG, with the goal of determining its approximate origin (see Chapter 3). This could help address one of the issues highlighted above: the lack of detailed studies on transient ALDFGs. Care should, however, be taken during replication studies, since the environment and surrounding temperatures would likely dictate the growth rates of different bivalve species and these factors would need to be replicated in the laboratory for accuracy. Depth plays a critical role on the rate of biofilm build-up since nets at deeper depths (beyond the epipelagic zone) are not exposed to as many macro-fouling organisms and the rate of build-up is subsequently reduced (Lehaitre et al., 2008). Also, there is a marked reduction in light penetration and effects from weather are minimized at these depths. These factors may contribute to the net remaining relatively physically unchanged for long periods of time at deeper depths. Humborstad et al. (2003) suggested that ghost fishing in deeper water could be a more serious problem since the only factor that appears to affect deep water ALDFG's ability to ghost fish is the amount of catch which weighs the net down, reducing its vertical height until it reaches the sea floor.

Multifilament nets, especially trawl nets, are made from thick synthetic materials that are buoyant in seawater. Attached floats give this type of ALDFG even more positive buoyancy. Over time, biofilm accumulation combined with the weight of catch causes the net to lose vertical profile and it sinks slowly (Macfadyen et al., 2009). It has been suggested that biofouling and catch may be flushed from the net during stormy weather (Ayaz et al., 2006). Coupled with the loss of ghost catch from predation, the nets could become buoyant again, rising back to the surface to continue the ghost fishing cycle (Figure 1.1).
Materials and methods

Here, I have carried out an extensive literature review with the aim of highlighting the threat of entanglement in ALDFG to marine megafauna. Furthermore, I tried to identify what gear poses the greatest threat to these animal groups.

Electronic keyword searches were performed using Google Scholar and Science Direct to identify literature. Keywords used, included: entanglement, ghost fishing, ghost gear, derelict fishing gear, marine debris, and ALDFG. These were paired with snake, crocodile, turtle, shark, ray, manta, seal, sea lion, whale, dolphin, manatee, dugong, pinniped, cetacean, elasmobranch, marine mammal, reptile, and megafauna. In addition, all cited references from each paper reviewed were extensively searched for keywords described above. Contact by email was made to the secretariat of the Global Ghost Gear Initiative (GGGI) and its working group members to identify additional sources of literature, particularly unpublished grey literature.

The Global Ghost Gear Initiative (GGGI), formed by World Animal Protection, is a cross-sectoral alliance committed to driving solutions to the problem of lost and abandoned fishing gear worldwide.
Laist (1997) compiled a comprehensive list of animal interactions with marine debris, which also included entanglements in fishing gear. Since this publication, new literature on the effects of marine debris has been published, with some focusing specifically on ghost fishing and others aimed more towards scarring that could not be attributed to ALDFG or interactions with fisheries (e.g. Robbins and Mattila, 2001, Bradford et al., 2009, Neilson et al., 2009, Knowlton et al., 2012). From this point on, I only discuss literature published between 1997 and 2015 and direct readers to Laist's (1997) review for data before this date.

Where possible, I screened the literature to focus on megafauna entanglement in ALDFG only. Most reports grouped general marine debris, such as plastic wrapping, clothing, cement bags, tires, metal rings, etc., with ALDFG. Combining these two entanglement hazards makes assessing ghost fishing entanglement rates and the effects they have on particular species or populations almost impossible to interpret. Furthermore, conclusions on the impact of ALDFG to various species are difficult to evaluate if the entangling material cannot be identified. To address this problem, I decided to implement a strict criterion during the selection of literature. I separated the number of animals entangled in ALDFG, or entanglements that could not be identified as being from active or derelict fishing gear, from those animals entangled in general marine debris, and those that were entangled during active fishing activity. If a clear separation was not possible then the number of animals entangled was not included in this chapter; this was to ensure that general marine debris entanglement or bycatch were not included in my interpretation.

1.4 Results

1.4.1 The effects of ghost fishing on marine mammals, reptiles and elasmobranchs

In total, 76 articles were identified, and 40 different species were recorded entangled in ALDFG (27 marine mammals’ species, seven reptile species, and six elasmobranchs species). Marine mammals accounted for 70% of all entanglements reported in this chapter. Humpback whales (Megaptera novaeangliae) were the most recorded species with 670 entangled individuals, closely followed by the North Atlantic right whale (Eubalaena glacialis, n = 648). Many observations of cetaceans were only of the tail (peduncle), which is left with scarring once the gear is eventually shed (e.g. Wells et al., 1998, Robbins and Mattila, 2004, Johnson et al., 2005, Jemison, 2009, Neilson et al., 2009). Humpback whales and North Atlantic right whales received considerable attention in the literature, in both instances accounting for 12% (total 24% for both species) of all entangled animals identified in this
In some studies, approximately half (48 - 57%) of the humpback whales assessed showed signs of some form of prior entanglement through photographic evidence (Robbins and Mattila, 2004). Similarly, when 626 photos of North Atlantic right whales from the East coast of USA and Canada were assessed, 83% showed evidence of entanglement (Knowlton et al., 2012). The highest number of entangled individuals for all pinnipeds on record was for the Antarctic fur seal (Arctocephalus gazelle, n = 492), followed by the Californian sea lion (Zalophus californianus, n = 443). The manatee (Trichechus manatus latirostris) was the fifth most recorded marine mammal entangled in ghost gear, despite only one published study being available (n = 375; Adimey et al., 2014; Table 1.1). Reptiles accounted for 27% of all animals entangled. With the exception of one saltwater crocodile (Crocodylus porosus), entangled in Australia, all reptiles recorded were sea turtles. The olive ridley turtle (Lepidochelys olivacea) accounted for the majority of identified sea turtles (68%, n = 303; Table 1.2). Only 2% of all entangled animals were elasmobranchs; however, the leafscale gulper shark (Centrophorous squamosus) and the Greenland shark (Somniosus microcephalus) were recorded by weight not by count (6.2 tons and 1 ton, respectively) and were, therefore, not included in the analyses of entangled individuals in this review (Table 1.3).

Since Laist's review in 1997, a total of 12 new species have been reported entangled in ALDFG in published or grey literature (Tables 1.1, 1.2, and 1.3). Unfortunately, Laist, (1997) did not quantify the number of individual animals entangled in ALDFG in his review; therefore, I cannot analyse changes over time in entanglement rate for particular species. Secondly, this review set a strict criterion to ensure general marine debris was not included in the results, while Laist, (1997) focused on marine debris as a whole, making direct comparisons between this review and Laist's difficult.
Table 1.1 Number of marine mammals recorded entangled in ghost gear since Laist (1997). (UR) Unknown Rope, (NL) Net Line combination, (ML) Monofilament Line, (RPT) Rope attached to Pots and Traps, (N) Nets.

<table>
<thead>
<tr>
<th>Species (nt = Total # of individual species entangled)</th>
<th>Ocean Basin</th>
<th>n</th>
<th>UR</th>
<th>NL</th>
<th>ML</th>
<th>RPT</th>
<th>N</th>
<th>Source</th>
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<tr>
<td>Grey seal (Halichoerus grypus) (nt = 262)</td>
<td>Atlantic</td>
<td>58</td>
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<td>204</td>
<td>8</td>
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<td></td>
<td>76</td>
<td>Sayer et al., 2015</td>
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<td>Guadalupe fur seal (Arctocephalus townsendi) (nt = 3)</td>
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<td>Moore et al., 2009</td>
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<tr>
<td>Antartic fur seal (Arctocephalus gazelle) (nt = 492)</td>
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<td>441</td>
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<td></td>
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<td>5</td>
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<td>375</td>
<td></td>
<td></td>
<td>286</td>
<td>83</td>
<td>4</td>
<td>Adimey et al., 2014</td>
</tr>
<tr>
<td></td>
<td>Indian</td>
<td>R</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Wilcox et al., 2014</td>
</tr>
<tr>
<td></td>
<td>Indian</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Gunn et al., 2010</td>
</tr>
<tr>
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<td>0</td>
<td>286</td>
<td>83</td>
<td>5</td>
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</tr>
<tr>
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<td></td>
<td>3834</td>
<td>44</td>
<td>822</td>
<td>175</td>
<td>669</td>
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</table>

* Species not reported in Laist, 1997 review
R = Literature review.

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<thead>
<tr>
<th>Species (nt = Total # of individual species entangled)</th>
<th>Ocean Basin</th>
<th>n</th>
<th>UR</th>
<th>NL</th>
<th>ML</th>
<th>RPT</th>
<th>N</th>
<th>Source</th>
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<td>Barreiros et al., 2014</td>
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<td></td>
<td></td>
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<td></td>
<td></td>
<td>3</td>
<td></td>
<td></td>
<td>Lopez-Jurado et al., 2003</td>
</tr>
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<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>Casale et al., 2010</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>163</td>
<td></td>
<td>163</td>
<td>Stelfox et al., 2015</td>
</tr>
<tr>
<td></td>
<td></td>
<td>163</td>
<td></td>
<td>53</td>
<td>25</td>
<td>44</td>
<td>44</td>
<td>Wilco et al., 2013</td>
</tr>
<tr>
<td></td>
<td></td>
<td>163</td>
<td></td>
<td>25</td>
<td>44</td>
<td></td>
<td></td>
<td>Anderson et al., 2009</td>
</tr>
<tr>
<td></td>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td>Jensen et al., 2013</td>
</tr>
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<td>163</td>
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<td></td>
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<td></td>
<td>163</td>
<td></td>
<td>53</td>
<td>25</td>
<td>44</td>
<td>44</td>
<td>Wilco et al., 2013</td>
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<td></td>
<td>163</td>
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<td>25</td>
<td>44</td>
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</tr>
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<td>18</td>
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<td>White, 2006</td>
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<tr>
<td>Green Turtle (<em>Chelonia mydas</em>) (nt = 16)</td>
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<td>2</td>
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<td>Stelfox et al., 2015</td>
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<td>Hawksbill Turtle (<em>Eretmochelys imbricata</em>) (nt = 43)</td>
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<td>6</td>
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<td></td>
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<td>Stelfox et al., 2015</td>
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<td></td>
<td></td>
<td>6</td>
<td></td>
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<td></td>
<td>White, 2006</td>
</tr>
<tr>
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<td>Indian</td>
<td>2</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td>Stelfox et al., 2015</td>
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<td></td>
<td></td>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td>White, 2006</td>
</tr>
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<td></td>
<td>Indian</td>
<td>35</td>
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<td></td>
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<td></td>
<td>Wilco et al., 2013</td>
</tr>
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<td></td>
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<td>35</td>
<td></td>
<td></td>
<td></td>
<td>Wilco et al., 2013</td>
</tr>
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<td>Leatherback Turtle (<em>Dermochelys coriacea</em>) (nt = 3)</td>
<td>Indian</td>
<td>1</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>Stelfox et al., 2015</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td>Stelfox et al., 2015</td>
</tr>
<tr>
<td></td>
<td>Indian</td>
<td>2</td>
<td></td>
<td></td>
<td>1</td>
<td></td>
<td></td>
<td>Moore et al., 2009</td>
</tr>
<tr>
<td>Flatback Turtle (<em>Natator depressor</em>) (nt = 3)*</td>
<td>Indian</td>
<td>3</td>
<td>3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Wilco et al., 2013</td>
</tr>
<tr>
<td>Saltwater crocodile (<em>Crocodylus porosus</em>) (nt = 1)*</td>
<td>Indian</td>
<td>1</td>
<td>1</td>
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<td></td>
<td></td>
<td></td>
<td>Gun et al., 2010</td>
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<tr>
<td>Unknown species and literature review (nt = 1041)</td>
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<td>13</td>
<td></td>
<td></td>
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<td>17</td>
<td>23</td>
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<td>Adimey et al., 2014</td>
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<td>2</td>
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<td>Nelms et al., 2015</td>
</tr>
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* Species not included in Laist, 1997 review,
R = Literature review

Total number of reptiles 1487 0 0 4 0 547
Table 1.3 Number of elasmobranchs entangled in ghost gear and multiple species reviews since Laist (1997). (UR) Unknown Rope, (NL) Net Line combination, (ML) Monofilament Line, (RPT) Rope attached to Pots and Traps, (N) Nets.

**Ghost gear type**

<table>
<thead>
<tr>
<th>Species (n_t = Total # of individual species entangled)</th>
<th>Ocean Basin</th>
<th>n</th>
<th>UR</th>
<th>NL</th>
<th>ML</th>
<th>RPT</th>
<th>N</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leafscale gulper shark (<em>Centrophorus squamosus</em>) (6.2t)<strong>(n_t = 6.2t)</strong></td>
<td>Atlantic</td>
<td>6.2**</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>6.2**</td>
<td>Large et al., 2009</td>
</tr>
<tr>
<td>Greenland shark (<em>Somniosus microcephalus</em>) (n_t = 1t)**</td>
<td>Atlantic</td>
<td>1**</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1**</td>
<td>Large et al., 2009</td>
</tr>
<tr>
<td>Nurse shark (<em>Ginglymostoma cirratum</em>) (n_t = 2*)</td>
<td>Atlantic</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2</td>
<td>Lopez-Jurado et al., 2003</td>
</tr>
<tr>
<td>Small tooth sawfish (<em>Pristis pectinata</em>) (n_t = 12)*</td>
<td>Atlantic</td>
<td>12</td>
<td>1</td>
<td>7</td>
<td>3</td>
<td></td>
<td>1</td>
<td>Seitz et al., 2006</td>
</tr>
<tr>
<td>Spiny dogfish shark (<em>Squalus acanthias</em>) (n_t = 103)*</td>
<td>Pacific</td>
<td>103</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>103</td>
<td>Good et al., 2010</td>
</tr>
<tr>
<td>Six gill shark (<em>Hexanchus griseus</em>) (n_t = 1)*</td>
<td>Pacific</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1</td>
<td>Good et al., 2010</td>
</tr>
<tr>
<td><em>Carcharhinus spp.</em> (n_t = 1)</td>
<td>Indian</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1</td>
<td>White, 2006</td>
</tr>
<tr>
<td><strong>Total number of elasmobranchs</strong></td>
<td>All</td>
<td>119</td>
<td>1</td>
<td>0</td>
<td>7</td>
<td>3</td>
<td>108</td>
<td></td>
</tr>
</tbody>
</table>

Review all species
Review all species
Review all species
Review all species
Review all species
Review all species

* Species not included in Laist, 1997 review.
** Number of entangled individuals recorded by weight in tons (t) not count. This data is excluded from all graphs
R = Literature review
1.4.2 Gear type

Just over half the ALDFG could not be identified to type because the animal either shed the gear before being found or the incident was recorded from photographs of scarring. 55% \( (n = 1324) \) of identified ALDFG were ghost fishing nets and 35% \( (n = 833) \) were monofilament lines (ML) externally entangling the animal. Ropes from traps and pots (RTP), unknown rope (UR), and a combination of net and line (NL) collectively accounted for only 10% of entangling gear. However, ML and RTP were the most observed gear types entangling cetaceans \( (n = 106 \) and \( n = 80 \), respectively; Figure 1.2). Of the 12 reptile publications that could identify gear type, only two reported monofilament line and the rest reported ghost nets as the primary entangling material. Ghost nets are highlighted in this chapter as being one of the major types of ALDFG affecting pinnipeds (56% of all recorded pinnipeds were entangled in nets) and sea turtles.

![Figure 1.2](image.png)

**Figure 1.2** Percentage of gear type entangling reptiles, pinnipeds, cetaceans, sirenia and elasmobranchs. (UR, black diamonds) Unknown Rope, (NL, black squares) Net Line combination, (ML, vertical lines) Monofilament Line, (RPT, horizontal lines) Rope attached to Pots and Traps, (N, solid black) Nets.

1.4.3 Bias in results

A distinct geographical bias was noticed in the literature reviewed. Together, the Atlantic \( (n = \)
35) and Pacific Oceans \((n = 18)\) dominated the research efforts, totalling 79% of all studies (excluding review papers), with the Indian \((n = 11)\), Southern \((n = 2)\), and Arctic Ocean \((n = 0)\) having considerably fewer published studies. This variation may be due to logistical difficulties combined with a lack of resources in these areas (Figure 1.3).

![Figure 1.3](image)

**Figure 1.3** Number of publications on ghost gear and megafauna entanglement between 1997 - 2015. Electronic keyword searches were performed using Google Scholar and Science Direct to identify literature. Keywords used, included: entanglement, ghost fishing, ghost gear, derelict fishing gear, marine debris, and ALDFG. These were paired with snake, crocodile, turtle, shark, ray, manta, seal, sea lion, whale, dolphin, manatee, dugong, pinniped, cetacean, elasmobranch, marine mammal, reptile, and megafauna

There appeared to be no correlation between the number of publications for a species and the recorded number of entangled individuals (Figure 1.4). For example, entangled humpback and North Atlantic right whales appeared in the most publications and also had the highest number of recorded entangled individuals compared to all other species. However, manatees, leafscale gulper sharks, and Greenland sharks appeared in very few published reports (one for each species) but an equally high number of entangled individuals were recorded. Spatial distribution between species could lead to a bias in the number of animals found in any given area (i.e. if more animals of a particular species reside in a particular habitat then it would make
sense to assume that that species has a higher chance of entanglement over a species rarely found in the same area). If we take this to be true, then the total number of entangled animals may be influenced by survey effort in that region; however, further work needs to be conducted to confirm or deny this hypothesis. It is also unlikely that all entanglement cases were recorded. An unknown number of entangled individuals never make it back to shore or are never observed and are therefore, less likely to be recorded (Fowler, 1987).
1.5 Discussion

1.5.1 Cetaceans and ghost fishing

The analysis of recent literature (from 1997 onwards) highlighted that all animal groups considered in this chapter are vulnerable to ghost fishing to some degree. The group most commonly recorded as entangled in the literature was cetaceans. However, this may be the result of observer bias, as positive identification of an animal’s interaction with ALDFG can only be made when the animal is observed at the surface or stranded on shore. Many observations of cetaceans were only of the tail (peduncle), which is left with scarring once the gear is eventually shed. However, for cetaceans, this means that another source of data (video and photographs) can be used to collect information on previous entanglements. One particular species that is of particular concern is the North Atlantic right whale because of its small population. The International Union for Conservation of Nature (IUCN) Red List of Threatened
Species classes the North Atlantic right whale as Endangered (IUCN, 2013) with an estimated population of only 526 individuals (Pettis and Hamilton, 2014). Entanglement in fishing gear is, therefore, a primary threat to this small population.

Johnson et al. (2005) concluded that fixed gear such as lobster pots and set gill nets were the main source of entanglement for whales, a result that is supported by this chapter. The ropes and lines routinely associated with this gear can easily entangle passing whales; however, we still do not know exactly how much of this gear, once lost or abandoned, interacts with passing whales. As discussed previously, it is difficult to attribute with certainty, the source of scarring on a cetacean to ALDFG or active fishing gear (e.g. Robbins, 2009, García-Godos et al., 2013).

Juvenile cetaceans appear to be the most at risk of mortality due to entanglement in comparison to adults (e.g. Zavadil et al., 2007, Cassoff et al., 2011, Knowlton et al., 2012, Lyman, 2012). Analyses of peduncle scars on adult humpback whales in Alaska and the Gulf of Maine suggest that the majority of individuals were entangled at some stage in their life (Robbins and Mattila, 2004, Neilson et al., 2009). However, when comparing the number of individuals that display scarring to the number of reported entanglements (which is low), it is tempting to speculate that larger animals are capable of either shedding gear or a large number of entanglement cases in these regions have gone unrecorded.

1.5.2 Pinnipeds and ghost fishing.

Pinniped-ALDFG interactions have been widely studied (e.g. Hanni and Pyle, 2000, Page et al., 2004, Allen et al., 2012, Sayer, 2015, etc.). Most entanglements occur around the neck or the body of the animal, which reduces their foraging capabilities, eventually leading to strangulation and starvation (Fowler, 1987). Entanglement likely increases drag as the animal moves through the water, further tiring them. Juvenile animals are more often observed entangled in marine debris (e.g. Henderson, 2001, Waluda and Staniland, 2013), which may be a reflection of the curious and playful behaviour of younger individuals (Fowler, 1987; Laist, 1997). Recent observations of juvenile grey seals interacting with fragments of monofilament and multifilament fishing net on shore may support this hypothesis (Allen et al., 2012). Female pinnipeds usually give birth to one pup annually and maternal care may last anywhere between four days to three years (Boness and Bowen, 1996). Supplying milk to pups is labour intensive; if females are entangled in ALDFG it may severely affect their haul out capability and ability to care for pups.

The reviewed studies that focused on pinnipeds and ALDFG highlighted three vulnerable
species: the Antarctic fur seal, the Californian sea lion, and the grey seal. Grey seals in the British Isles account for around 39% of the world's population and recent research has suggested that the grey seals living on the coast of Wales and SW England are a genetically distinct sub-population of around 5000 individuals (Allen et al., 2012). These facts together raise the concern of the consequence of ALDFG to this sub-population, in particular.

Although we can attempt to quantify the effects of entanglements on certain populations of pinnipeds, estimating mortality due to ghost fishing directly remains difficult because most publications for this group recorded all marine debris items, including plastic packaging, rubber bands, plastic sheet, cloth, six pack holders, etc. together with ALDFG (e.g. Zavadil et al., 2007, Waluda and Staniland, 2013).

1.5.3 Manatee and dugong and ghost fishing

Only three publications documented the effect of ghost fishing or ALDFG on manatees and dugongs, but one study did record a large number of entangled manatees ($n = 375$; Adimey et al., 2014). As with most species covered in this chapter, it is difficult to make any conclusions about the effect ALDFG has on manatee populations, due primarily to the lack of research directed on this species. Manatees occupy both freshwater and coastal marine environments and sport fishers encroach on both environments, increasing the chance of this species' interaction with derelict gear. Globally, there has been an increase in sport fishing and results from Adimey et al. (2014) show that monofilament lines and hooks are the main debris that manatees interact with (either through ingestion or entanglement). However, there is not enough evidence to suggest that hook and line is the only significant type of ALDFG posing a risk to this species.

1.5.4 Marine turtles and ghost fishing

The group with the second highest entanglement rate in this chapter and the focus of the rest of this thesis was reptiles, in particular, marine turtles. Marine turtle-ALDFG interactions are of particular concern because ALDFG presents a significant problem in three key turtle habitats: foraging grounds, breeding grounds and the open ocean. ALDFG on nesting beaches may act as obstacles for both nesting females and hatchlings. Hatchlings may become entangled preventing them from ever making it into the sea (Carr, 1987, Ramos et al., 2015). Furthermore, wind and sand movements may bury ALDFG over time, resulting in nesting females digging into net material (Pers. Obs.). Monofilament fishing nets (in particular), pose a significant risk to sea turtles on coral reefs as monofilament nets are very thin and they are
likely undetectable to the turtles (Pers. Obs. in Maldives and Pakistan). ALDFG floating in the open ocean are also of particular concern, as sea turtles spend their first three to five years floating with oceanic currents and many species migrate long distances across the open ocean between breeding and foraging grounds. It is likely that during this time they come into contact with marine debris that follows the same currents (Carr, 1987). Floating algal mats on the sea surface act as an ideal substrate for sedentary animals, such as hydrozoan, bryozoan, and barnacles, to attach and start growing. ALDFG behaves in the same way as these algal mats. Animals, such as turtles, are often attracted to these floating mats or ALDFG in search of shelter and food (Carr, 1987). Of the seven species of sea turtle that exist globally, two species are known to spend more of their adult years in oceanic waters: the olive ridley (*Lepidochelys olivacea*) and the leatherback (*Dermochelys coriacea*). Other species are opportunistic feeders during their juvenile life stage but switch to benthic feeding in neritic zones as they move into adulthood (Carr, 1987). Therefore, leatherbacks and olive ridley turtles probably have a higher chance of encountering floating ALDFG, as they inhabit the open ocean for more of their life cycle compared to other turtle species. However, this review reports a disproportionally larger number of olive ridleys entangled in ghost nets than leatherbacks and this could be explained by the difference in abundance between the two species. For example, the largest rookery of olive ridleys can be found along the east coast of India consisting of around 200,000 nesting individuals (Manoharakrishnan and Swaminathan, 2018) Comparatively the largest leatherback rookery is estimated to be much lower at around 40,000 in Gabon, South Atlantic (Witt et al., 2009). Therefore, it would be reasonable to assume that olive ridleys have a greater probability of encountering ALDFG and becoming entangled.

One of the few studies that addressed turtle entanglement in ALDFG was a spatial risk analyses conducted by Wilcox et al. (2013). The authors combined oceanic drift and beach clean data with known distributions of turtle species in Australia in order to determine entanglement locations and level of risk. Results highlighted that entanglements occurred in areas with both high ghost net density and high turtle density. Stranding records where turtles were observed dead or alive offered a good opportunity to conduct further research and validate Wilcox et al.’s (2015) model predictions (see also Chapter 4). Two further studies, this time in Brazil (Casale et al., 2010 Santos et al., 2012) found that the majority of olive ridley turtles found entangled were sub-adults or adults. Nelms et al. (2015) suggested that this could be because juveniles have a greater chance of escaping the ALDFG as they are smaller or that nets were impacting breeding or migrating areas and not impacting juvenile habitats. Conversely, recent
data on entangled olive ridley turtles in the Maldives found that the majority of entangled turtles were juveniles; however, the reason for this remains unclear and needs to be explored in greater detail (Stelfox and Hudgins, 2015). This chapter clearly highlights the dangers of ghost nets in comparison to other ALDFG for marine turtles (see Chapters 2 and 4).

1.5.5 Elasmobranchs and ghost fishing.

This group of organisms appears to be either less vulnerable to entanglement or (and more likely), fewer studies have assessed the level of impact of ghost fishing on these organisms. Despite the fact that only a few studies have been published on ghost fishing and elasmobranchs, relatively high numbers of entangled sharks and rays were noted. It may be the case that certain species are more vulnerable to ALDFG than others. For example, the unique elongated, toothed rostrum of the small tooth sawfish (*Pristis pectinata*) likely puts this species at higher risk of entanglement in ghost nets. Although not directly fitting into the remit of this chapter and the thesis as a whole, bycatch of sawfish is said to be the probable cause of declining populations of this species, which has resulted in the sawfish being assigned Critical Endangered status by the IUCN (IUCN, 2013). The direct effect of ALDFG on shark populations remains unknown due to the lack of data. However, the limited number of studies that have been undertaken with a focus on shark species suggests that ALDFG could be a significant cause of mortality. Top predators, such as sharks, are known to be attracted to floating mats of algae or debris as these mats are home to many of their prey, who in turn are in search of shelter and food. Indeed, the fishing industry actually exploits this phenomenon, with purse seine and pole and line fisheries using artificial floating devices known as Fish Aggregating Devices (or FADs) to attract their catch. FADs usually consist of a floating frame with a marker on the surface and long aggregators (nets) hanging underneath. While attracting their target species, such as tuna, many other non-targeted species are also caught. Studies have shown that silky sharks (*Carcharhinus falciformis*) and oceanic white tip sharks (*Carcharhinus longimanus*) together make up around 90% of the bycatch from this type of fishing (Gilman, 2011). One of the dangers of FADs is that once they are lost, they continue to fish at full potential (i.e. ghost fish). The Indian Ocean Tuna Commission (IOTC) are aware of this problem and that it can undermine the efficiency of conservation and management measures that they have put in place. However, retrieval of FADs in the Indian Ocean is un-regulated. We do know that ghost fishing of silky sharks has impacted abundance and catch rate, however the true effect that the purse seiners have had and continue to have on this species in particular is likely to be severely underrepresented (IOTC, 2013).
1.5.6 Management and mitigation

Numerous tactics have been implemented to address the issue of ghost gear and it has been suggested that the problem can be approached in two ways: 1) using preventative or 2) curative measures (Brown and Macfadyen, 2007). Arguments can be made for and against both methods and it is evident that gear retrieval is the main curative tool practiced globally (e.g. Guillory, 2001, Brown and Macfadyen, 2007, Large et al., 2009). In deep water, ghost gear can be recovered using “creepers”; machinery that is towed behind fishing boats. The Norwegian creeper is one such example. It is a three-metre-long bar with three dredges attached by a hinge. It is used to scrape the seabed and snag any abandoned nets (Large et al., 2009). Other creeper designs exist but they tend to apply the same principles of snagging. This method is only effective when a certain level of prior knowledge is available. This includes where the proposed gear lies, the amount of gear, the bottom topography, and the presence of any sensitive habitats. Bad weather can prevent retrieval attempts altogether. Furthermore, a study by Cefas (2006) noted that no full gill nets were ever recorded when this practice was undertaken. This begs the question whether creepers are actually capable of retrieving full nets or if the creeper simply rips through the entire net only retrieving pieces at a time. To my knowledge, no reliable in-water observations exist to confirm or contradict this hypothesis. Gear retrieval on shallow reefs and wrecks requires a different method of retrieval. Often divers are used in such instances, as done by various NGOs such as Ghost Fishing, Olive Ridley Project, North West Straits, Project Aware, etc. Limitations do, however, exist here as well, including dive limits, depth, bottom time, human safety (chance of entanglement), and weather conditions. Alternatively using biodegradable materials in fishing gear design may help minimise the effect of ghost fishing from lost gear (Wilcox and Hardesty, 2016). For example, biodegradable nets (Kim et al., 2016) speed up the deterioration of the net structure which leads to minimal ghost fishing over time. Similarly, biodegradable trap doors on pots and traps helps ghost fished species escape which breaks the continues ghost fishing cycle (Bilkovic et al., 2012).

Regardless of the method of removal, it has been suggested that detailed cost-benefit analyses should be conducted with regard to measuring the effectiveness of different methods of dealing with ALDFG. This would assist in identifying what methods are most effective, which could help navigate managerial decisions (Gilardi et al., 2010). In addition to the removal of ALDFG, the importance of education of fishers must also be mentioned. For instance, a fisher incentive
program to deposit old or damaged nets at designated collection points was first met with resistance in South Korea, but, after educational workshops, the program quickly became a success with Incheon City collecting 18,000 tons of derelict gear in only four years (Cho, 2005). However, it was also highlighted that this program may have stopped if no further financial support was given for fishers to dispose of nets correctly. Similarly, in northern Australia a combination of building trust, providing resources, and building capacity for rangers by actively listening and giving feedback to indigenous communities, resulted in the recovery of 5532 ghost nets. Of these, 45% were identified back to their original source fishery (Gunn et al., 2010), which allowed for preventative measures to be put into place.

Identifying where fishing gear was initially lost is a particularly important challenge facing the study and management of ALDFG. Finding ghost gear in vast oceans can be difficult but research has shown that various sensors, such as video, thermal imaging, and radar used in manned or unmanned aircraft can be effective tools to locate ALDFG (Pichel et al., 2012). Alternatively, Mace (2012) suggested that using sensors to detect eddies and convergence zones in the open ocean may be a more effective way to find ALDFG conglomerations. However, sensors can be expensive and their deployment subject to weather conditions. Furthermore, it is recognised that sensors cannot perform all necessary steps from detection to removal (e.g. Mace, 2012, Pichel et al., 2012). Datasets obtained from Lagrangian drifters have helped to identify marine debris accumulation hotspots over time (Maximenko et al., 2012) (see Chapter 3); however, although this is a useful tool for retrieval operations, it does not help to identify where the ALDFG initially came from. Drifters also have a very limited battery life and are rarely deployed from coastal regions (Martinez et al., 2009). The same study noted that geostrophic currents and Ekman drift have the ability to influence ALDFG drift patterns. For example, two tagged Dungeness crab traps deployed from the state of Oregon were eventually recovered at two separate locations in the NW Hawaiian Islands four years later (Ebbesmeyer et al., 2012). Ocean Surface Current Simulator (OSCURS) models, combined with estimated loss dates acquired from interviews of the crab pot owners, allowed for potential drifting paths to be created. This is a good example of how numerical data can be used to identify drift patterns. In addition, it highlights the importance of gear marking in making precise identification of ALDFG back to its relevant fisheries. More research into detachment from buoy rate, vertical profile, and rates of fouling (see Chapter 3) are needed to understand the effect that these may have on drifting patterns (McElwee and Morishige, 2010). Furthermore, the drifting patterns of floating ALDFG may be subject to winds, currents, and
weather and the exact effects that these have on drifting ALDFG is still unknown (McElwee and Morishige, 2010). Tagging ALDFG may help confirm the accuracy of predictive models.

Incorporating fishing communities into ghost gear management strategies has proven to be an effective method when managing the ghost gear issue. For example, understanding the causes of fishing gear loss helped identify why fishing gear is lost which can help identify possible intervention (Richardson et al., 2018). Similarly, extracting knowledge from fishers in Norway helped build evidence to generate a sustainable management of fishing gear to avoid gear loss (Deshpande et al., 2019). Many NGOs (for example; Ghost diving (www.ghostdiving.com) and Olive Ridley Project (www.oliveridleyproject.org) rely on fishing communities to help report and recover ghost gear by teams of divers. This helps build trust between different stakeholders and minimise the impact of ghost fishing from ghost gear.

1.6 Conclusions

Though much literature exists on entanglement of many animal species in marine debris, linking ALDFG specifically to these entanglements (versus active fishing gear or general marine debris) is a relatively new field of study with very few published articles focused on this topic. There is a strong need for more research into the effects of ghost fishing at the population level and more focused research that explores preventative solutions. It was therefore apparent that additional research be directed to the Indian Ocean (and hence the direction of this thesis - see Chapters 2, 3 and 4), along with the Southern, and Arctic Oceans. Any work in these areas would address the gaps in data, which currently exist. This chapter also highlighted that improved identification of entangled animals to species level is also required. Carefully separating ALDFG from general marine debris during data analyses could help accurately quantify the problem for each of these species. Interestingly, the majority of studies I assessed during this chapter estimated entanglement rates by combining general marine debris with ALDFG or by looking at scarring in photos. It would be useful if ALDFG gets treated separately from general marine debris, as fisheries need a different managerial approach when compared to debris originating from passing tourist or cruise boats. Further this chapter identified the difficulties of indicating the effects of ALDFG on marine organisms and the majority of observations rely on the animal bringing the entangling material back to land,
the animal being sighted entangled at sea, or analysing scars on the animal's body to identify entanglements. Moreover, it is not always possible to make the distinction between interaction with ALDFG and interaction with active fishing gear, especially in larger animals, such as cetaceans. In instances where fishing gear can be distinguished as the cause of entanglement, comparisons should be made to existing data from fishing interactions to roughly determine the number of animals interacting with ALDFG versus those interacting with active fishing operations. Education of fishers and observers is an important first step to increase this type of data recording.

During research for this review it became clear that entanglement records for all species are scattered and sparse. Many institutes, biologists, diving groups, NGOs, and local governments have information on ghost fishing incidences, but these incidences are rarely published, and the issue is likely underrepresented in published literature. Many institutions are working independently to collect this information; however, regional databases for each major ocean basin and records of entanglements for each major animal group in those regions would provide consistent data and greatly improve knowledge on ALDFG interactions. Data collection in the future should follow a minimum set of global criteria, which would help direct future studies and allow for comparisons to be made in order to identify hotspot areas for ALDFG production and marine life interactions. It would also help direct effort and focus funding to particular sites.
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Appendix

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Appendix 1.1 Rebuttal to Review in Chapter 1.


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Appendix 1.2 Response to Rebuttal by Asmutis-Silvia et al. 2017.

**Chapter 2** Untangling the origin of ghost gear within the Maldivian archipelago and its impact on olive ridley (*Lepidochelys olivacea*) populations.

2.1 Abstract

There is little documentation available on the impact that abandoned, lost or discarded fishing nets (‘ghost nets’) have on turtle populations. Here I collected data on ghost net and turtle entanglements in the Maldivian archipelago over a 51 month period to assess (a) if a particular net type or characteristic was identifiable as entangling more turtles and (b) if particular fishing practices (i.e. the types of nets) could be potentially managed to reduce turtle entanglement in the Maldivian archipelago. A total of 131 turtles were entangled in the 752 reported ghost nets, and olive ridley turtles (*Lepidochelys olivacea*) appeared to be the most vulnerable (making up 97% of entangled turtles). However, this study estimates that the 752 nets reported over a 51-month period could entangle between 3,400 and 12,200 turtles across the Indian Ocean prior to detection in the Maldives. The logistic regression and random forest models identified mesh size, seasonality (i.e. north east monsoon), and the presence of floats as variables significantly affecting the likelihood of turtle entanglement. The probability of entanglement increased as the log mesh size increased but decreased when floats were present. Additionally, turtles were more likely to be entangled during the north east monsoon when currents flow from east to west. The Partitioning Around Mediods (PAM) cluster analysis indicated that there were at least 11 broadly assigned net types found floating in the study area and these were dominated by trawl and gill nets. The analysis highlights the need for a detailed database of existing gear types coupled with gear marking to improve traceability of ghost nets in the Indian Ocean.
2.2 Introduction

Human activity is thought to impact every ecosystem on Earth (Halpern et al., 2008, Cardinale et al., 2012). One mechanism of impact that is receiving increasing attention is that of marine litter (Fossi et al., 2018, Karthik et al., 2018, Krishnakumar et al., 2018, Vlachogianni et al., 2018, Sweet et al., 2019). It is estimated, that abandoned, lost or discarded fishing gear (ALDFG or ‘ghost gear’) make up approximately 10% of this litter (Macfadyan et al., 2009). Ghost gear, specifically ghost nets can have major implications on ecosystems and their biodiversity, entangling a vast array of species in a continuous process referred to as ghost fishing (Laist et al., 1995). Sea turtles are particularly vulnerable to this type of litter (Wilcox et al., 2016, Stelfox et al., 2016, Duncan et al. 2017). Although non-target captures (bycatch) of sea turtles in active fishing gear has been studied and shown to have significant impacts on turtle populations (Bourjea et al., 2008, Wallace et al., 2010, Peckham et al., 2016, Lucchetti et al., 2017), the scale of impacts due to ghost nets are less understood.

It is likely that the life history characteristics of turtles make them particularly vulnerable to entanglement in ghost nets. For example, sea turtles are poikilotherms (i.e. they cannot regulate their body temperature), hence the need to bask on beaches (Whittow and Balazs, 1982) or particularly in the case of juveniles, floating algal mats have been shown as a suitable basking substrate (Nichols et al., 2001). Ghost nets may therefore act as an alternative floating substrate and an attractive option for turtles seeking to bask, resulting in increased risk of entanglement. Juvenile sea turtles are also often attracted to these floating algal mats (and therefore also the floating nets) as a source of food and shelter (Boyle et al., 2008). Comparatively foraging sub-adult and adult olive ridleys near Fernando de Noronha and Atol das Rocos in Brazil were reported entangled in ghost nets (Santos et al., 2012). This provides evidence to suggest that
all life stages of sea turtles are at risk of entanglement. Moreover, a survey of experts rated entanglement as a greater threat to sea turtles than climate change, oil pollution and direct exploitation (Duncan et al., 2017).

Although ghost nets are a global issue, geographic differences in ocean currents, climatic conditions and local fishing practices are likely to generate spatial heterogeneity in the impact on turtle populations (e.g. Gulf of Carpentaria, Northern Australia, Gunn et al., 2010). This is further complicated by the high degree of regional connectivity between ocean borders. For example, a genetic based study on olive ridleys (Lepidochelys olivacea) caught in ghost nets around Australia highlighted a variety of different, and mostly unidentified, genetic lineages or haplotypes (Jensen et al., 2013), indicating ghost nets impact several genetically distinct rookeries spanning large geographical ranges (Jensen et al., 2013). In the Indian Ocean there remains a sparsity of knowledge on turtle populations and the effects ghost nets have on these (see Chapter 4). Throughout this region, there is considerable spatial heterogeneity in the use of fishing gear. For example, purse seine fishing and the use of drifting Fish Aggregating Devices (dFADs) dominate throughout much of the Western Indian Ocean (Davies et al., 2014), and pelagic gill nets and trawling are commonly utilised throughout the Arabian and Bay of Bengal ecoregions (Dar et al., 2017, Thomas et al., 2017, Jones et al., 2018, Samanta et al., 2018). An additional source of complexity affecting the distribution of ghost nets in the Indian Ocean is the complex weather patterns, driven by two major components; the north east (NE) monsoon (where surface geostrophic currents generally flow westward between November - April), and the south west (SW) monsoon (where surface geostrophic currents generally flow eastward between May – October; Shankar et al., 2002).

The construction of reliable statistical models relating environmental conditions, regional fishing practices, and patterns in ocean currents to turtle entanglement in ghost nets, would enable the design of more effective tools for management and mitigation of this threat to turtle
populations. However, the cryptic and transboundary nature of ghost nets, the limited knowledge associated with environmental conditions at the time of entanglement and the identities of the fisheries that lose the gear in the first place, make such models difficult to construct. Indeed, studies which have tackled such questions (but based on the effects of bycatch and turtle entanglement) utilise temporal data (Casale et al., 2017) combined with knowledge of the fishery responsible. For ghost nets, this information is simply unknown. As such, to date, only one study has attempted to do this (Wilcox et al., 2015). This study indicated that larger mesh sizes and lighter twine (indicative of pelagic gill nets), increased the probability of entanglements in the gulf of Carpentaria, Australia. They then used their model to extrapolate the scale of the issue and hypothesised that over 14,600 turtles would have been caught in the 8,690 nets sampled in their study, if these nets drifted for only one year (Wilcox et al., 2015). Statistical modelling assessing entanglements are limited in the available literature, therefore new methods need to be explored to fill in these data gaps and extend our understanding of the impact of plastics in general. For example, a risk analyses approach using logistic regression was used to provide, for the first time, a global assessment of plastic ingestion by sea turtles of different species across different life stages (Schuyler et al., 2014). Similarly, scat-based molecular diet analyses and quantification of ingested microplastic were combined to assess the dietary exposure of wild grey seals (Halichoerus grypus) to microplastics (Nelms et al., 2019). Unfortunately ghost net data and associated entanglements are complex, statistical tools must be able to handle imbalanced, skewed, mixed data and complex interactions between dependant variables. At present there is a lack of available literature on the impact that ghost nets have on sea turtles and even fewer studies that can be used as a baseline for statistical modelling.

Here, I aim to utilise ghost net and turtle entanglement data collected over five years by the Olive Ridley Project (ORP) across 26 atolls spread over roughly 90,000 square kilometres in
the Maldivian archipelago. Statistical tools related to modelling the probability of turtle entanglement will then be used to analyse the data collected. The geographical position of the Maldives (centred in the Indian Ocean) offers a rare opportunity to assess the spatial dynamics of ghost nets through regional connectivity. As fishing by nets is limited to bait fishing (in the Maldives) particularly associated with the tuna fishery (Miller et al., 2017) and these nets have a small mesh size and are rarely found as ghost nets in the Maldives (Pers. Obs.). Therefore I assume that a large proportion of the floating ghost nets in this region originate from neighbouring countries or within the exclusive economic zone of the Maldives through illegal, unreported or unregulated fishing activity (Chapter 3). Additionally, the atolls in the Maldives draw a perpendicular line across the direction of ocean currents, with the atolls and outer reefs acting as traps for floating debris (Stelfox et al., 2015). This therefore acts as a ‘natural’ sampling filter. Here, I built on the study by Wilcox et al. (2015) by developing a model in a new geographical area and including aspects of seasonality (NE and SW monsoon) as potential factors affecting the probability of turtle entanglement. Furthermore, I investigated the applicability of a novel approach of clustering net characteristics to identify groupings of gear types found drifting into the Maldives.

2.3 Materials and Method

There are several statistical methodologies available for relating independent variables (in this instance characteristics of the nets or environmental factors) to the probability of a turtle entangling with a ghost net, all of which have comparative strengths and weaknesses. In this study, I therefore utilised a combination of methods (logistic regression, random forests and Partitioning Around Mediods (PAM) clustering; detailed below). These methods were chosen
as they have been extensively used in several research fields, are well understood mathematically, and represent a transition from traditional statistical methods, through machine learning, to exploratory methods. Additionally, they are relatively robust to outliers and can be effectively applied to smaller datasets.

The Olive Ridley Project (ORP) is a UK charity which collects data from citizen scientists on ghost net and turtle entanglements found in the Indian Ocean. Here I report on data that has been collected in the Maldives between July 2013 – September 2017 (51 months in total) across 26 atolls stretching over 90,000 square kilometres. Ghost net characteristics \((n = 752, \text{ north east } = 437, \text{ south west } = 315)\), thought to be potential factors affecting the probability of turtle entanglement, were collected opportunistically following a protocol developed by the International Union for Conservation of Nature (IUCN) and ORP (see Appendix 2.1 for detailed information on net characteristics collected by citizen scientists and analysed in this study) (https://oliveridleyproject.org/report-a-ghost-net). All ghost nets were reported by citizen scientists when finding them on beaches or on their way to dive or snorkelling sites, floating on the surface or entangled on shallow coral reefs inside the atolls of the Maldivian archipelago (see Appendix 2.2 for location of ghost nets entangling sea turtles). The location and date of net discovery, and whether a turtle was entangled or not (with a species identification if known) were also recorded. Photographs accompanied each turtle entanglement to help validate species identification, those with no photographs were excluded from further analyses. Moreover, only whole carcases were recorded in this analysis, skeleton and bones were excluded because species could not be accurately identified. The majority of ghost nets were burnt or sent to landfill after data collection with a small proportion recycled into bracelets. A second independent dataset \((n = 49\) nets) was also collected in the same way, but over a shorter period (September 2017 and January 2018). The second dataset was utilised as independent data for testing predictions derived from the model (random forest, explained
below) developed using the first dataset. This independent dataset did not overlap the study period of the first dataset and nets were incinerated after data collection.

2.3.1 Logistic regression

Logistic regression used the generalised linear model (GLM) framework with a logit link function and binomial error distribution (Equation 1). This relates explanatory variables (ghost net and environmental characteristics) to a binary response variable (entanglement, no entanglement) (McCullagh, 1984). Thus, here the link function relates effects of net characteristics and seasons to the probability that a net will contain an entangled turtle. The link function is usually expressed in the form of the natural logarithm (log) of the odds (of a net containing a turtle) (see Equation 1).

\[
\log \left( \frac{\pi}{\pi - 1} \right) = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \cdots + \beta_i x_i
\]

Equation 1. Expression of the logit link function used in a logistic regression equation. The left-hand term is the logit link, with \( \pi \) being the probability of an event (entanglement) occurring. Therefore, the logit form is the log of the odds of an event occurring. The right-hand side is the standard linear regression form showing the effects (\( \beta \) coefficients) of each of the explanatory variables (\( X_1, X_2 \ldots X_i \)) on the log of the odds. \( \beta_0 \) represents the expected log odds value when all independent variables have value zero.

Two of the independent variables, mesh size (min = 10mm, max = 9700mm, mean = 128.49 mm) and twine diameter (min = 0.1mm, max = 15mm, mean = 1.65mm) had very skewed distributions and were therefore log transformed prior to performing logistic regression. I added
1 to twine diameter before logging to keep values within the domain of the log function due to some being close to zero in the first instance.

An information theoretic approach (Akaike Information Criterion (AIC); Akaike, 1981) was used to select the minimum adequate model. This approach is regarded as being a more robust selection method than the use of likelihood ratio tests (Burnham et al., 1995). Both forward and backward stepwise selection was used to ensure consistency in selection. Moreover, a comparison of residual deviance and degrees of freedom indicated no problems with over or under dispersion.

2.3.2 Building classifiers with rare events

Random forests were chosen as a classification method as they are known to perform well on complex non-linear data, have few statistical assumptions, are relatively robust when dealing with missing data and automatically incorporate interactions in their construction (Faraway, 2016). Such models have been utilised in similar studies of bycatch (Báez et al., 2014, Oliver et al., 2015, James et al., 2016). For these reasons, our random forest was selected as our final predictive model and used to calculate the probability of turtle entanglement.

The choice of hyperparameters used in the construction of random forests can alter model output, with studies indicating that the number of parameters randomly sampled at each split in the tree (mtry) is the most influential hyperparameter improving performance (see Probst and Boulesteix, 2018, for a review). In this study, mtry was tuned based on a sequential range between 1 and 20 (20 representing the full set of independent variables). When using the same cross validation sets to tune model hyperparameters and estimate model performance, optimistically biased estimates are known to occur (Cawley and Talbot, 2010). To overcome this bias, a nested cross validation was conducted which allowed for hyperparameter tuning in the inner loop and an estimate of the overall model generalisation to be made on the outer loop
(Varma and Simon, 2006). This ensured that no data was ‘leaked’ from the test set during model tuning. Inside both loops a stratified k-fold cross validation (k = 10) was performed. This allowed minority and majority cases to be present in each random fold. A combination of $F_1$ score and sensitivity (tpr) and specificity (tnr) measures were utilised to assess the applicability of hyperparameter values and to evaluate model generalisation:

The best random forest classifiers (those most likely to generalise well on unseen data) were selected as those giving the highest $F_1$ score, closest to 1. Sensitivity and specificity were then used as additional metrics to quantify rates of false negatives and false positives, respectively.

All analyses were conducted using the statistical programming language R vs. 3.4.2 (R Core Team, 2018). Random forest classifiers were constructed using the ‘randomForest’ package (Liaw and Wiener, 2002), with variable selection done using the ‘cforest’ function in the ‘party’ package (Hothorn et al., 2006). The cforest algorithm does not show bias when a mix of categorical and continuous variables are used, unlike tradition variable importance measure from the random forest (Strobl et al., 2008). k-fold cross validation (k = 10) was performed within the ‘caret’ package (Khun, 2018). Variables used in the final random forest model were selected based on starting with ten of the highest-ranking variables (according to the cforest function), and then removing one variable at a time until the highest $F_1$ score was achieved.

2.3.3 Dealing with rare events

Rare events, such as ghost net entanglements, are a challenge when building classifiers. Many algorithms perform poorly with such data, typically resulting in the minority class (here turtle entangled) being ignored (King and Zeng, 2001). It is possible to use ‘over-sampling’ (replicating observations from the minority class) and ‘under-sampling’ (removing observations from the majority class) to try and minimise the impact of the rarity of the event (Estabrooks and Japkowicz, 2004). However, over-sampling can result in bias in the selection
of the minority class, whilst under-sampling can result in a loss of important data (Weiss, 2004). An alternative to these more traditional methods is the synthetic minority over-sampling technique (SMOTE). This takes minority class observations and generates synthetic ones. These are based on randomly positioning ‘new’ values of the minority class along a Euclidean path within multi-parameter space, thereby joining pairs of locations of actual observations of the minority class (Chawla et al. 2002). SMOTE was used in this study on the random forest classifiers to adjust the class imbalance in order to ensure that there were equal numbers of entanglement and no-entanglement cases.

2.3.4 Assigning fishing nets to fisheries

Partitioning Around Medoids (PAM) was utilised to create clusters of net characteristics (Kaufman and Rousseau, 1990). PAM works in a similar way to k-means clustering but uses representative objects (here particular nets) as ‘centres’ of clusters. This contrasts with utilising locations in multi-dimensional space that do not correspond with a particular object. Medoids are identified based on minimised mean dissimilarity of an object and the other objects in the corresponding cluster. This has the effect of reducing the sensitivity of cluster designations to outliers. Dissimilarity distances were based on the Gower dissimilarity index to allow for mixed variables. Distances were calculated using the ‘daisy’ function in the ‘cluster’ package (Maechler et al., 2017). The average silhouette width (Rousseau, 1987) was utilised to assess the validity of the PAM clustering. Silhouette width is a measure of how similar an object is to its own cluster. Values range between -1 and +1 and a high value indicates that the object is well fitted to its cluster.

PAM clustering can be susceptible to finding local maxima causing the preferential influence of categorical variables. To avoid this problem, I evenly weighted the numerical variables (mesh size and twine diameter) more heavily than the categorical variables and adjusted these
evenly until the silhouette width reached a local maximum. The ’construction’ variable was also weighted to allow monofilament fishing nets, rarely found in the Maldives, to influence the clustering. Categorical variables were treated as nominal values in the calculation of the Gower indices, and the two continuous variables (stretched mesh size and twine diameter) were treated as ratio scaled.

2.4 Results

A total of 1069 ghost net fragments, entangling 7 green (*Chelonia mydas*), 18 hawksbills (*Eretmochelys imbricata*), 1 leatherback (*Dermochelys coriacea*), 348 olive ridley (*Lepidochelys olivacea*) and 3 unidentified sea turtles were reported. Due to limitations in the field not all ghost nets could be measured. Therefore, 752 ghost nets fragments were analysed in total, of which 80 had one or more turtle(s) entangled (Figure 2.1). A total of 131 sea turtles were entangled in the 752 reported ghost nets, olive ridley turtles made up 97% of turtles caught. A further 49 ghost nets entangling 7 olive ridley turtles were reported between September 2017 and January 2018. These latter data were utilised as the test set to assess the performance of the final predictive regression tree model. For more information on the ghost nets typically found in the Maldives please refer to Stelfox et al. (2015).
Figure 2.1. Frequency distribution of the total number (n = 752) of ghost nets found in the Maldives over 51 months between July 2013 – September 2017. During the north east monsoon (November – April) 437 ghost nets were analysed. Comparatively, the south west monsoon (May – October) had 315 ghost nets analysed.

2.4.1 Logistic regression

Stepwise regression analyses identified mesh size, seasonality (i.e. NE monsoon), and presence of floats as variables significantly affecting the probability of turtle entanglement (Table 2.1). However, the estimated fit of the model was poor (0.055; pseudo-R\(^2\) following McFadden, 1979).
Table 2.1 Estimated regression coefficients for the minimum adequate logistic regression model, estimating the probability of a net having captured a turtle. Results are from a stepwise selection using the Akaike Information Criterion.

<table>
<thead>
<tr>
<th>Coefficients</th>
<th>Estimates</th>
<th>Std. Error</th>
<th>Z value</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
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<td>0.673</td>
<td>-5.979</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>Logmesh</td>
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<td>0.122</td>
<td>2.738</td>
<td>0.006</td>
</tr>
<tr>
<td>NE monsoon</td>
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<td>0.256</td>
<td>2.219</td>
<td>0.027</td>
</tr>
<tr>
<td>Floats</td>
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</tr>
<tr>
<td>Blue</td>
<td>0.489</td>
<td>0.280</td>
<td>1.743</td>
<td>0.081</td>
</tr>
</tbody>
</table>

The probability of entanglement increased as the log mesh size increased (Figure. 2.2) but decreased when floats were present. Additionally, the model indicated that turtles were more likely to be entangled during the NE monsoon when currents flow from east to west. Despite net colour (blue) having a marginally insignificant positive impact, this trait was still included in the model as the model had the lowest AIC value (348, with maximum AIC values of other models ranging up to AIC 368.49), and we took a conservative approach given the complexities of the structure of the data.
Figure 2.2 Estimated effect of net mesh size on the probability of turtle entanglement (solid blue line) from the logistic regression model. Black rugs at the top of the graph represent entanglement events, and rugs on the bottom represent no entanglement events. The positions of the rugs along the x-axis mark the log of the mesh size for the corresponding net. The grey band represents the 95% confidence interval for the estimated probability.

2.4.2 Random forest model predictions

Adjusting for class imbalance using SMOTE greatly improved the random forest model ($F_1 = 0.24$ compared with $F_1 = 0.04$). Model generalisation was best when variables were reduced to the top six ranking variables (Figure 2.3) ($F_1 = 0.26$, $tpr = 0.66$, $tnr = 0.58$).

Variable selection for the random forests generally complimented the final logistic regression model structure. However, twine diameter was judged to be an additional influential variable (Figure 2.3).
Figure 2.3 Ranking (decreasing order) based on the influence of dummy variables on the random forest model. NE = North East Monsoon, SW = South West Monsoon, S1-S5 = number of strands (1 - 5), net colour (blue, green, black, white and rare colours (yellow, orange and red), mesh size (mm), twine diameter (mm), net construction (braided, multifilament, monofilament) and material (synthetic or natural). Excluding Monofilament, S4, S1, White, S2, Braided, Synthetic, Natural, Multifilament and S5 as variable importance is equal to zero.

A systematic grid search approach using the top six ranking variables did not show any convincing trends in effects on $m_{try}$ selection (Appendix 2.3). Searching for the optimal value for $m_{try}$ was inconclusive, and I used a value of three, based on it resulting in the highest $F_1$ score ($F_1 = 0.30$).
The final model (6 top ranked variables and m\text{try} = 3), when applied to the test data set, generalised to a similar extent as that of the model in the developmental stage (f1 = 0.32). The low sensitivity (tpr = 0.57) and higher specificity (tnr = 0.66) demonstrate that the models are sensitive to type I errors. However, I was able to capture 63% of ‘true’ events. Although this was at the expense of an increase in the number of false positives (Figure 2.4).

![Confusion matrix](image)

**Figure 2.4** Confusion matrix of final model on unseen data (f1 = 0.32). Red numbers indicate correctly identified observations. The proportion of false positives was 0.29 (i.e. 14/48).

### 2.4.3 Ghost net clustering

The diversity of ‘types’ of net used within the Indian Ocean region is high. However, the average silhouette width gave statistical support for 11 apparent net clusters (0.65) amongst the 752 ghost net fragments analysed in this study (Appendix 2.4).

Eight of these 11 clusters had greater average silhouette width meaning that these 8 clusters are well assigned and likely to reflect true variation in net types. In contrast, the remaining three clusters (8, 10 and 11) showed incorrect or poorly assigned observations, which
subsequently reduced the average silhouette width (Appendix 2.5). While the heterogeneity within these 3 clusters was not large enough to warrant the addition of a cluster according to the average silhouette width, all their respective medoids had characteristics, which were distinct from those of the other clusters (Table 2.2). For example, cluster 11 had only 1 observation which gave a negative silhouette width, and its characteristics are unlikely to be associated with any net in the Indian Ocean. Further, nets \((n = 13, \text{Table 2.2})\) in cluster 8 have far heavier twine than the other clusters and, in contrast, nets in cluster 10 \((n = 54)\) had a larger mesh size but light twine.

The bulk of observations were in the first two clusters and included most of the ghost nets which were predicted to have high probabilities of entangling turtles. Clusters showed a large overlap in mesh sizes making it unlikely that individual clusters could be linked to specific fisheries (Appendix 2.6). For example, the heterogeneity in clusters 1, 2, 4, 6, 7 and 10 meant that the clustering did not involve a clear distinction between gill and trawl nets that these clusters likely belong to. However, the clustering did lead to some broad separations of net types based on net characteristics.

Table 2.2 Medoid characteristics showing the number of observations \((n)\) assigned to each cluster. Turtle entanglements are shown as the total number of turtles entangled in each cluster (actual). Number of turtles predicted by the random forest before type 1 error correction (predicted), and the number of turtles predicted entangled after the type 1 error correction (adjusted - 29%).
Discussion

2.5.1 Causes of turtle entanglement

The results of this study indicate that turtle entanglement is more likely to occur as the mesh size of ghost nets increases. This supports other studies conducted across Northern Australia (Wilcox et al., 2015) and the US mid-Atlantic (Murray, 2009). While the latter study focused on bycatch and not ghost net entanglement, this illustrates the importance of mesh size in relation to the entanglement of turtles on a global scale. In the present study, the majority of ghost nets encountered were largely damaged fragments, likely torn away from the main body of the net. Damaged small meshed nets may have an altered mesh size or large openings which
could become problematic for sea turtles. Wilcox et al. (2015) highlighted that thinner twine was more likely to entangle turtles. Although this was not captured by my logistic regression, it was identified as a possible factor in the random forest analysis. Therefore, I suggest that although twine diameter does not appear to be a dominant variable explaining turtle entanglement it remains important for building predictive models, at least in this instance. Here we also highlight that ghost nets found during the NE monsoon showed a significantly greater probability of having entangled turtles in them compared to ghost nets found in the counter currents generated from the SW monsoon. This supports previous studies, which highlight the importance of seasonality and turtle entanglements in active fishing gear (Murray et al., 2004, Tomás et al., 2008, Kot et al., 2009). In the Indian Ocean, the NE monsoon coincides with peak nesting season for olive ridley turtles along the east coast of India (Pandev et al., 1997). Therefore, it is reasonable to assume that this results in an increase in turtle entanglement.

Surprisingly, ghost nets with no floats attached appeared to be more likely to entangle turtles. This contradicts studies focusing on active fishing gear and turtle entanglement, where they found the opposite trend and which has led to the suggestion of decreasing or removing floats from active fishing gear to reduce the probability of entanglement (Echwikhi et al., 2010, Gillman et al., 2010, Peckham et al., 2016). It could be hypothesised that the added buoyancy with floats may keep turtles at the surface for longer, giving them more time to escape. However, further work is needed to understand why this difference occurs and if management decisions need to take this into account.

Blue netting was also identified by the random forests as being a high-ranking variable and was marginal in the logistic regression. Turtles have a well-developed sense of vision that clearly plays an important part in foraging behaviour (Swimmer et al., 2005, Southwood et al. 2008). However, most of our knowledge on sea turtle vision has derived from studies of loggerhead (*Caretta caretta*) (Narazaki et al., 2013, Piovano et al., 2013) and green turtles
(Mäthger et al., 2007). In contrast, no information on the visual capabilities for olive ridley turtles exists. However, it remains unknown if olive ridleys are more attracted to blue nets than other colours, or if blue nets remain invisible given their lack of contrast against the blue of the ocean. Interestingly, Schuyler et al. (2012) created model sea turtle visual systems and used it to analyse debris from beach surveys and necropsied turtles. The authors found that turtles ate fewer blue items suggesting that this may be because blue plastics are less visible against the blue background of the ocean. This study corroborates with findings by Ryan et al. (2016) that found more white and translucent items in necropsied post hatchling loggerheads than blue items. This is the first time that a possible link between turtle entanglement and ghost net colour has been made. However, comparable studies of plastic ingestion by sea turtles may support this link. However, the exact form and mechanism of the relationship remain to be identified, and further research needs to be conducted to explore this.

Interestingly, in this study I estimate entanglement rates of 0.17 (131/752) however in the most comparable study (Wilcox et al., 2015), rates were much lower (0.02%). It is uncertain why the Maldives has significantly higher entanglement rates but given the Maldives proximity to one of the largest aggregations of olive ridleys in the world (Shanker et al., 2004) this could be one major driver to increased probability of entanglement.

2.5.2 Impact on sea turtles

The predictive models (random forest) estimated that 168 turtles \((n = 236\) non-adjusted models) were entangled across all 752 ghost nets found during the length of this study. Although this number seems inflated when compared to the actual number of entangling events \((n = 80)\), it is important to consider that this study only recorded ghost nets at the end of their life, and therefore I was unable to account for those turtles that managed to escape or decomposed prior to net stranding. Reports of turtle remains in ghost nets are not uncommon in the Maldives.
(Figure 2.5) but there is little information on the decay rate of turtles in this region. Research in other areas of the tropics suggests that turtles may decay rapidly in only 5 to 18 days (Santos et al., 2018). With a decay rate of 5 days, if we assume that on average nets drift for 1 year and turtles are evenly distributed across the region where ghost nets drift, then the portion of turtles entangled that could be detected would be 0.0137 year$^{-1}$ (5 d/365 days). Therefore, based on the predicted 168 turtles entangled this suggests that ~12,200 turtles may have been entangled in the nets recovered over the length of this study (51 months). With a decay rate of 18 days, this drops to ~3,400 turtles. These calculations are based on assuming an average behaviour of ghost nets, but it is important to recognise that there is likely to be considerable temporal and spatial heterogeneity in the probability of encountering a net with an entangled turtle prior to the completion of decay. If we are to more accurately estimate entanglement probabilities and the true numbers of turtles being entangled, then two key parameters need to be quantified much more accurately: decay rates in the Indian Ocean and the actual number of ghost nets in the oceans at any particular time. Moreover, the random forest models have a relatively high false positive rate (0.29). The dataset was imbalanced, particularly with respect to the number of entanglements compared with the number of non-entanglements. Random forests are known to produce high false positive rates, so in order to address this, I used SMOTE. However, although this undoubtedly improved the situation, some impacts of imbalance may remain, as SMOTE is still not a perfect tool.
Figure 2.5 Humerus bone of an unidentified species of sea turtle in the Maldives. Evidence of prior entanglement. Photo credit: Claire Petros

5.3 Potential origin of nets

There is a general pattern of concern from artisanal fishers that an increase in competition and conflict is leading to a loss of catch (Bennett et al., 2001, Whitmarch et al., 2003). As a result, artisanal fishers have become increasingly opportunistic and carry multiple gear types during a single fishing trip. By doing so they can target different species and sizes which maximises fishing effort (Samoilys et al., 2017). A better understanding of the effects of ghost nets on turtle populations requires a much better knowledge of how many of the active nets become ghost nets. However, gaining such knowledge and identifying the origins of ghost nets are very difficult with the diversity of fishing gear design being utilised at local scales. Further, the
complexity of ocean currents and surface movement driven by wind direction makes identifying ghost nets origins considerably more difficult (see Chapter 3).

The PAM analyses could not cluster net types according to fisheries, and this could be explained by the similarities in gear type across fisheries and countries. For example, multifilament gill nets targeting seer fish (*Scomberomorus* sp.), mackerel (*Rastrelliger kanagurta*) and pomfret (*Brama* sp.) in Mumbai, India typically have light twine and mesh size ranging between 70 – 150 mm (Dar and Thomas, 2016). In contrast, drifting gill nets used to target *Caranx* sp. in Sri Lanka have mesh sizes ranging between 150 – 450 mm (Thivviyan and Jayakody, 2017). Therefore, in theory, these two fisheries should have separated out in the analysis. However, in Maharashtra, India, gill nets targeting pomfret, mackerel and seer fish have light twine and mesh sizes ranging between 40 - 280 mm. Similarly, trawl fisheries in Andhra Pradesh, India, have been reported to have a mix of net types, ranging between 20 mm at the cod-end to 2000 mm at the mouth of the net (Rajeswari et al., 2012). These overlaps within and between fishery types will affect the ability of statistical methods to cluster net types according to fisheries. The same issues apply when trying to identify the geographical origin of the ghost nets. For example, if I aimed to ascertain where in the Indian Ocean ghost nets are coming from, I would need to be able to differentiate between major fishing districts. However, these fisheries predominantly use the same gear type. For example, surveys of fishing net types used in two different districts in India (Maharashtra for gill nets (Nirmali et al., 2007) and Kerala for trawl nets (Sayana et al., 2016) both report mesh sizes ranging between 20 - 200 mm. However, certain patterns and trends could be discerned from the data. For example, most nets reported in cluster 3 were dark braided nets. Upon closer inspection, fragments of bamboo were regularly associated with these nets (Pers. Obs.) and these are consistent with dFADs deployed predominantly in the western Indian Ocean operated by fishers from the EU (Balderson and Martin, 2015). These nets are found in varying conditions and occasionally
trackers have been found to be attached, allowing for identification of responsible fisheries supporting these conclusions (Stelfox et al., 2015). Further, by adjusting weights in the model I was able to cluster rarely seen nets such as monofilament gill nets that are occasionally found in the Maldives (as in cluster five). As highlighted above, these types of nets are widely utilised across many fisheries and many countries in the Indian Ocean. However, few were found in the surveys throughout the Maldives, and few of those had entangled turtles within. This may be because the density of nylon (the material commonly used to make monofilaments nets) is 1.14 g cm\(^{-3}\), meaning that it will sink in seawater. It is important to note that, although this study indicates that these nets are unlikely to be a major threat of entanglement, this study is not suggesting that this implies any reduction in their effects on other marine life. Indeed, these nets are more likely to affect local fishing grounds and it would not be surprising if an increase in these nets occurred close to operation of these fisheries. Critically, the use of nets, other than for the bait fishery, is banned in the Maldives. However, illegal fishing does occur, a result likely due to lack of enforcement (Pers. Obs.). It may therefore be the case that some of the nets reported in this analysis have actually been utilised in the Maldives after all.

2.5.4 Solutions and mitigation: Gear traceability

Traceability of ghost nets was one of the major goals of this study. However, the complexity of usage of gear types used by fishers suggests that this is unlikely to be possible. The FAO have recognised that gear marking is a valuable method to improve traceability of ownership of gear types (FAO, 2019). Moreover, methods such as barcoding, gear modification, radio frequency identification (RFID) and global positioning systems (GPS) can help improve traceability of lost fishing gear (He and Suuronen, 2018). However, these methods require significant human resources and can be expensive. One possible solution that may complement existing gear marking methods would be to utilise Blockchain technology (Swan, 2015) to improve traceability. Blockchain is a decentralised, immutable and distributed ledger that
offers a transparent method to store data (Probst, 2019). The blockchain would allow traceability of fishing nets from manufacture right the way through to the fisher at the end of the supply chain. Each transaction is stored as a “block” and each new successive block makes up the blockchain ledger. It is reported that blockchain technology can handle this type of complex relationship very well (Swan, 2016) and it is already being piloted to improve traceability of tuna caught in the Pacific Islands (Visser and Hanich, 2017). Utilising such technological advances may therefore help close the gap on understanding what type of gear is being sold and where, identify high net sales, problem areas and potential red flags for certain gear types in a complex system with many stakeholders. Furthermore, the use of blockchain technology could be driven by consumer demand for more sustainable fisheries with greater accountability, leading to reduced gear loss associated with illegal, unreported and unregulated fishing activity.

2.5.5 Solutions and mitigation: Ghost net prevention

This study clearly highlights the need for an urgent reduction in the loss of net fragments associated with gill and trawl net fisheries. However, the difficulties of implementing and enforcing such a broad recommendation in the region means we may have to look at alternatives. An important first step would be to identify why gear is lost and develop solutions through information sharing between all stakeholders within the supply chain. Improved compliance on gear loss reporting by country members of the Indian Ocean Tuna Commission (IOTC), may help determine which fisheries are facing the greatest challenge with regards to gear loss, which may help reallocate resources to minimise the issue from these fisheries.

The importance of seasonality, with regard to determining the probability of entanglement, was highlighted by my model in this chapter. Therefore, there is an urgent need to identify migratory pathways of olive ridleys in the surrounding area to help pinpoint fishery overlap.
Temporary closures in these overlapping areas (during high turtle activity) may reduce the likelihood of turtle entanglement as a result of gear loss caused by operational damage or general discard. Moreover, establishing free, port side or landing site recycling facilities would discourage small and large-scale fishers from dumping damaged or end of life gear at sea. Given the large number of damaged and fragmented ghost nets reported in this analysis, buffer zones could be developed to ensure rugged bathymetric zones are avoided or controlled for, reducing gear damage and subsequent gear loss.

Finally, I have also demonstrated that net colour plays a role, albeit a relatively small one, in increasing the likelihood of sea turtle entanglement. However, more research will need to be conducted in order to understand this relationship before managerial decisions can be made on gear modifications surrounding net colour.

2.6. Conclusion

Although we cannot be sure how long ghost nets drift before being located within the Maldives (see Chapter 3 for more details on this), I have attempted to quantify the number of turtles entangled within this region and therefore highlight the level of threat faced by this one aspect of marine litter. Our results highlight that ghost net entanglement is likely a global issue and not a problem that individual countries will be able to deal with alone (see Chapter 3). Given the potential threat of ghost nets to sea turtles in the area, I recommend that bycatch models account for gear loss to ensure that the impact that fisheries have on sea turtle populations are not underestimated. Future research on this topic should focus on methods to age ghost nets, for example, bioaccumulation rates so that this information can be fitted into models such as those outlined in this study (see Chapter 3). This will allow us to include drift times allowing
us to get one step closer to identifying the source of individual nets and reliably estimating the number of turtles entangled over the lifetime of a net (as discussed in this Chapter). The clustering models highlighted the continued need of gear loss reporting to researchers, charities and governmental agencies, enabling existing evidence surrounding gear loss in the Indian Ocean to be gathered more effectively. There is an urgent need to advance our knowledge on gear types used by artisanal fishing communities. To this end, I recommend the construction of a detailed regional database of existing gear types so that positive identification of sources of ghost nets from artisanal fisheries may be possible. This could be coordinated by regional fisheries management organisations such as the Indian Ocean Tuna Commission (IOTC). Finally, future resources should be focussed on a combination of improved gear traceability and the implementation of realistic measures to decrease gear loss in order to more effectively reduce amounts of ghost nets.
References

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turtles (Caretta caretta) use vision to forage on gelatinous prey in mid-water. *PLoS One*, 8(6).


Santos, A.J., Bellini, C., Bortolon, L.F., and Coluchi, R.O., 2012. Ghost nets haunt the olive ridley turtle (Lepidochelys olivacea) near the Brazilian Islands of Fernando de Noronha


Appendix

Appendix 2.1 Ghost net data collected by citizen scientists between 2013 and 2017 in the Maldivian Archipelago (excluding turtle present/absent). Net characteristics were reported via an online data portal hosted on the Olive Ridley Project (ORP) website developed by ORP and the International Union for Conservation of Nature (IUCN) https://oliveridleyproject.org/report-a-ghost-net. For model building all variables were treated as dummy variables and split according to the number of levels.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Description</th>
<th>categorical/numerical</th>
<th>Levels</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Season</td>
<td>Split into North East Monsoon (NE Monsoon) - Currents flow from east to west (November - April) and South West Monsoon (SW Monsoon) currents flow from west to east (May - October)</td>
<td>Categorical</td>
<td>2</td>
<td>NA</td>
</tr>
<tr>
<td>Floats</td>
<td>Floats in the form of plastic bottles or bouys attached to the net.</td>
<td>Categorical</td>
<td>1</td>
<td>NA</td>
</tr>
<tr>
<td><strong>Net colour</strong></td>
<td>Net colour includes green, black, blue, white and rare (yellow, red and orange)</td>
<td>Categorical</td>
<td>5</td>
<td>NA</td>
</tr>
<tr>
<td><strong>Number of strands</strong></td>
<td>Number of fibres that make up a single twine. Scale 1-5</td>
<td>Numerical</td>
<td>5</td>
<td>1-5</td>
</tr>
<tr>
<td><strong>Material</strong></td>
<td>Nets were either made from natural fibres such as coconut and cotton and categorised as &quot;natural&quot; or made from synthetic fibres such as plastics and labelled &quot;synthetic&quot;</td>
<td>Categorical</td>
<td>2</td>
<td>NA</td>
</tr>
<tr>
<td><strong>Net construction</strong></td>
<td>Nets could be one of three categories; monofilament, multifilament or braided twine.</td>
<td>Categorical</td>
<td>3</td>
<td>NA</td>
</tr>
<tr>
<td><strong>Twine diameter</strong></td>
<td>Diameter of twine in mm Distance between two knots that make up the mesh.</td>
<td>Numerical</td>
<td>NA</td>
<td>0.1 - 15mm</td>
</tr>
<tr>
<td>Mesh size</td>
<td>Knotts stretched before</td>
<td>Numerical</td>
<td>NA</td>
<td>10 - 9700mm</td>
</tr>
<tr>
<td>-----------</td>
<td>-------------------------</td>
<td>-----------</td>
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<td>-------------</td>
</tr>
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</table>

measurements taken in mm

Sensitivity: Internal
Appendix 2.2 Ghost nets found during the south west monsoon (n = 315) (Top) and north east monsoon (n = 437) (Bottom) in the Maldivian Archipelago. Arrows represent general direction of surface ocean currents.
Appendix 2.3 Learner performance (mean $F_1$ score) plotted against the values of $m_{try}$. The analysis was performed within a nested cross validation with 10 nested cv runs (colour coded key). Explanatory variables included only the top 6 performing variables identified by the cforest; floats, monsoon season, mesh size, colour (blue) and twine diameter.
Appendix 2.4. Silhouette width against the number of clusters (ghost net clusters). Clusters identified based on minimised mean dissimilarity of a ghost net and the other ghost net in the corresponding cluster. The best number of clusters are determined by the highest silhouette width, in this case 0.65 which highlights 11 clusters best describe the data.
Appendix 2.5. Silhouette plot illustrating the 11 clusters found across all 752 ghost net samples assessed. The red dotted line represents the average silhouette width value (0.65). The width of each cluster represents a graphical representation of the number of nets assigned to that cluster. Clusters that show a negative silhouette width (<0.00) indicates incorrectly assigned observation.
Appendix 2.6. Mean mesh and twine sizes for each cluster (except cluster 11). Standard Deviation of each cluster is shown in the error bars.
Chapter 3. Minimum drift times infer trajectories of ghost nets found in the Maldives.

3.1 Abstract

Abandoned, lost or discarded fishing nets, more commonly referred to as ghost nets are a major cause of entanglement for marine animals. Identifying responsible fisheries is challenging, relying on prior knowledge of the fisheries and the gear types they are utilising in any given area. Alternatively, we can turn to ocean current simulations to estimate drift trajectories of ghost nets, however this method relies on estimated drift times in order to backtrack nets to a potential origin. This study explores a range of possible methods to estimate minimum drift times of ghost nets found in the Maldives with the aim of identifying a putative origin. I did this by analysing fouling organisms growing on the surface of fishing nets and surface buoys on fixed lines. I highlight that percentage cover of biofouling organisms and capitulum length of the gooseneck barnacles *Lepas anatifera* are two methods that provide estimations of water exposure time and subsequent drift times. I applied the two methods to age ghost gear on eight ghost nets collected in the Maldives and estimated age ranges between 7.5 to 101 days. I then used Lagrangian simulations to backtrack the putative origin of these eight ghost nets. I also simulated the drift of 326 historical ghost nets for which I had no estimates of drift duration. For these nets, I simulated their drift backwards from their collected locations and times using several values (10, 30, 60, 90 and 120 days) of drift duration. Purse seine and gill nets are found to be ‘high risk’ fisheries that overlap drift trajectories between 30 and 120 days, regardless of season for both the eight aged ghost nets and the historical ghost nets. However, ghost nets below 30 days remain inside the exclusive economic zone of the Maldivian archipelago highlighting the possibility of illegal, unreported and unregulated fishing activity in the area. This study highlights the urgent need for gear loss reporting by purse seine and gill net fisheries and improved data on fishery spatial distribution in the area from large- and small-scale fisheries.
Keywords

Ghost nets, larangian, purse seine, gill nets, biofouling, drift trajectories, plastisphere

3.2 Introduction

Abandoned, lost or discarded fishing gear (ALDFG) also known as ‘ghost gear’ has been widely recognised as one of the most important components of debris in our oceans (Watters et al. 2010, Hardesty et al., 2015, Stelfox et al., 2016, Wilcox et al. 2016, Consoli et al., 2018, Miller et al., 2018). Historically, the majority of fishing gear was made from natural materials such as cotton, coconut or hemp. Therefore, they would have had a relatively short lifespan even when lost or abandoned. However, starting in the late 1940s and, early 50s these materials were replaced with synthetics, extending their lifetime substantially (von Brandt, 1984). Now, when nets are abandoned, lost or discarded they often become locked in ocean gyres or travel great distances, crossing political borders before eventually becoming stranded or found in nearshore habitats like coastal coral reefs (Matsuoka, 2005, Stelfox et al., 2015). Management decisions on this issue are often challenging as ‘ownership’ of the gear is often unknown and difficult to backtrack.

Large quantities of ghost gear have been reported in the Maldivian archipelago (Stelfox et al., 2015, Stelfox et al., 2019), despite commercial application of pole and line for catching tuna and subsistence hand line methods dominating the fisheries in the Indian Ocean island state (Adam et al., 2015). Understanding where ALDFG are coming from would be a first step to tackling the issue and reducing the threats to marine life. To date, broad classifications of the possible fisheries responsible have been identified by statistically modelling ghost net characteristics and attempting to assign them to a specific fishery (Wilcox et al., 2013, Stelfox et al., 2019). However, aging the floating nets to provide time adrift, and analysing drifting
trajectories using ocean current simulations in combination with the spatial distribution of fisheries should bring us closer to identifying their origins.

There are a number of ways one can monitor and/or obtain drift times for floating debris. For example, the assessment of fouling organisms on debris (Hellio et al., 2004, Banerjee et al., 2011, Callow et al., 2011, Kiessling et al., 2015, Fazey et al., 2016). Typically, active fishing gear is cleaned between trips, effectively minimising damage and increasing longevity and catch efficiency (Pers. Obs.). Biological growth on the surface of nets would thereby act as a ‘biological clock’ indicating how long they have been in the water since they were last cleaned. Indeed, the succession of certain species or taxa and their respective growth rates have been shown to age other types of marine debris (Ye et al., 1991). In particular, diatoms and barnacles may be useful indicator groups to assess drift as they are prevalent across all oceans and are major biofouling organisms on all types of litter including nets (Saldanha et al., 2003, Magni et al., 2015). The majority of research has, to date, focused on ways of removing biofouling or preventing settlement in the first instance (Hellio et al., 2004, Banerjee et al., 2011, Callow et al., 2011). This is because biofouling can reduce efficiency on floating wave energy devices (Nall et al., 2017), block water intake pipes (Rajagopal and Jenner, 2012) for example, as well as adding weight to fixed structures (Shi et al., 2012), and increasing drag and subsequent fuel consumption in marine vessels (Schultz, 2007).

Another approach commonly utilised to predict the origin of marine litter (e.g. plastics) and drift trajectories in general is the use of ocean current data (Lebreton et al., 2012, Maximenko et al., 2012, Liubartseva et al., 2016, Guerrini et al., 2019, Jalón-Rojas et al., 2019). For example, dispersal patterns of post hatchling and juvenile leatherback sea turtles (Demochelys coriacea) were simulated by releasing particles that drift passively into a World Ocean circulation model (Gasper et al., 2012). The authors combined drift trajectories with spatial data on bycatch, genetics, sightings and satellite telemetry to reveal that juvenile survival is
largely driven by variable ocean currents and found that those drifting into the Indian Ocean likely suffer high mortality rates. Lagrangian drift models are another useful tool to identify drift trajectories and several studies have used these models to determine drift trajectories of post hatchling sea turtles. For example, over 280,000 particles were released near sea turtle nesting beaches in the Mediterranean to determine migration origin (Hays et al., 2010). Similarly, lagrangian simulations were assessed to understand trans-Pacific dispersal of loggerhead (*Carretta carretta*) sea turtle hatchlings by releasing 10,000 particles from each of the three nesting regions in Japan (Okuyama et al., 2011). Lagrangian drifters are often favoured in marine vertebrate tracking as they offer high temporal and spatial resolution (Fossette et al., 2012). However, it is important to understand that a high degree of variation in the correlation between satellite derived current estimates are observed along the paths of lagrangian drifters (e.g. Sudre and Morrow, 2008), therefore analyses of drift trajectories of object or animal must take this into account.

The Maldives provides an interesting case study as the country lies north south perpendicular to an east west current system (Shankar et al., 2002). However, ocean circulation is complex, and currents vary not only seasonally, but in response to short term events like tropical cyclones and between years depending on pan-tropical atmospheric-ocean forcing. This latter aspect is related to phenomena like the El Niño Southern Oscillation and the Indian Ocean Dipole (Schott et al., 2009). This means broad generalisation is not always appropriate when analysing the dispersal of objects (Wood et al., 2016).

In this study, we attempted to assess the origin of ghost gear found within the Maldivian archipelago using a multipronged approach. First, we experimentally deployed nets to quantify the development and growth rates of biofouling organisms in order to estimate time adrift. Measurements included diatom population and taxonomy and percentage cover of all fouling organisms. Additionally, we deployed surface buoys to analyse growth rates of a common
biofouling organism, the pelagic gooseneck barnacle (*Lepas anatifera*). Secondly, we applied these age estimate techniques to eight ghost nets found floating in the Maldives and backtracked the nets (based on these age estimates) using a Lagrangian model to find their putative origin. Here, we also utilised a historical and much larger dataset of 326 recovered ghost nets (each with reported times and locations of recovery in Maldivian waters but without estimates of their drift durations) to explore which other fisheries may be responsible for these ‘lost’ nets. To do this, we utilised Lagrangian modelling to backtrack their putative origin using several plausible values of drift duration (10, 30, 60, 90 and 120 days).

### 3.3 Methods and materials

#### 3.3.1 Study site

The Maldivian archipelago consists of 26 atolls stretching across almost 1 million square km (Adam, 2006). Geographically, the Maldives is enclosed along its northern border and lies north-south across an east-west monsoonal current system (Shankar et al., 2004, Appendix 3.1). Variation in sea surface temperature (SST) is very small in the Maldives typically ranging between 28.27 - 29.38°C (Alonso-Garcia et al., 2018). However, in this study SST ranged between 28 - 31°C, however consistent measurements were not taken throughout the study period.

All experiments were conducted inside atoll lagoon in shallow coastal waters, 18m deep and 20m away from the sloping reef (Appendix 3.2). In contrast ghost net fragments were collected opportunistically from the ocean surface from inside island atolls (lagoons) or from outside the atoll chain in deeper oceanic water (Appendix 3.3). Atoll lagoons in the Maldives vary in depth...
from 30 m to 50 m (Fritz et al., 2006) and are typically calmer more sheltered waters than deeper oceanic waters. The Maldives is subject to two major monsoonal patterns that bring with it opposing ocean surface currents. During the northeast (NE) monsoon, surface currents approaching the islands originate from the Bay of Bengal, whereas during the southwest (SW) monsoon they approach from the Arabian Sea (Shankar et al., 2004). Typically, floating debris, including ghost nets from neighbouring countries get trapped by the island chain. This gives researchers the unique opportunity to remove and analyse this debris to determine drift trajectories and putative origin.

3.3.2 Experimental assessment of bioaccumulation on floating nets and buoys

Diatom diversity and biofouling percentage cover were assessed on newly deployed, high-density polypropylene (HDPP), multifilament fishing nets (supplied by Garware Wall Ropes Ltd). This study was conducted at the Dhuni Kholu resort, in Baa atoll, Maldives (5° 2’27.17”N, 72°53’4.01”E). A single net was divided into three separate replicate fragments (100 cm X 100 cm size). Two fishing buoys were attached to the sea floor, 18 m above and 20 m away from the sloping reef. The two buoys were fixed by a further rope on the surface (Appendix 3.4). The nets were attached to the line with zip ties that were first coated with antifouling paint to minimise cross contamination. Each repeat (net) was arranged so that no direct contact could be made between replicates (Appendix 3.4). The nets were sub-sampled (ensuring the knot and twine either side was included – Appendix 3.5) on day two, four, six, eight and ten, then every two weeks thereafter up until 112 days.

Samples were placed immediately in 2.5% glutaraldehyde and stored in a fridge until further sample preparation and analysis. Samples were then dehydrated via a series of 60, 75, 85, 95 and 100% absolute ethanol for 15 minutes each, with final dehydration consisting of air drying for 1 hr. Specimens were then mounted on an aluminium stub with Achesons Silver Dag (dried
overnight) and coated with gold (standard 15nm) using an Emi Tech K550X Splutter Coating Unit. Specimens were then examined using a Stereoscan 240 scanning electron microscope, and digital images collected by Orion 6.60.6 software. Scanning electron micrographs (at a magnification of 1500 X) were taken along a 0.1 mm² transect in the middle of the twine and 0.1 mm² transect along the centre of the knot. We assessed each micrograph for diatoms, which were identified to the lowest taxonomic or morphological level. Diatoms that fell outside the transect frame or not attached to the net itself (i.e. likely associated with the surrounding water column) were excluded from the analyses. Moreover, diatoms that could not be identified due to partial loss of structure were also excluded. We then calculated commonly used diversity indices such as species richness ($S$ – total number of different species within the transect frame), total abundance, Shannon-Wiener diversity index ($H$, Shannon and Weaver, 1949), Simpson index ($D$, Simpson, 1949) and the Pielou evenness index ($J$, Pielou, 1966). These indices allowed us to explore species rarity, abundance and distribution for each time interval to identify patterns over time. We also opportunistically recorded additional organisms of note, in order to capture any successional changes of other biota communities over time. However, these were excluded from diversity analyses due to the difficulty in distinguishing between background noise (which may include filamentous algae, mucilage, encrusting communities and/or the high abundance of bacteria that would skew diversity indices for example). That said, we did combine diatom and other opportunistically recorded organism counts to perform a Z score hierarchical clustering using Euclidean distance measure. Here each row (organism or species) was scaled before analyses using heatmap.2 function in gplot (Warnes et al., 2014). This allowed for a visual representation of community succession.

Alongside diversity indices, percentage cover of all biota growing on the surface of the net was calculated. To do this, three scanning electron micrographs along each transect (as mentioned above) were imaged to give a 0.03 mm² surface area per micrograph (six micrographs per
sample, knot and twine). Images were then inputted into the digital imaging software, Image J (Schneider et al., 2012). The threshold of each micrograph was manually adjusted until attached biota were highlighted in red to estimate percentage cover. In rare cases, salt crystals were present on the surface. However, these were excluded from all analyses and we assumed no growth was occurring under the crystal. Due to contrast and brightness variations in some images not all of the biota was captured by the threshold adjustments and in some instances, threshold only captured a small proportion of the visible surface growth. In such cases, the paintbrush tool was used to manually highlight the cover.

The growth rate of gooseneck barnacles (*Lepas anatifera*) was measured in a separate experiment at the Bodu Hithi resort, North Male atoll, Maldives (4°25'41.18"N, 73°23'7.70"E). Three surface buoys, on a fixed line 20 m away from the sloping reef, 18 m above the sea floor were deployed. When biofouling had occurred, 27 gooseneck barnacles (9 for each of the three replicates/buoys) were randomly selected and their growth rates calculated. The capitulum length was measured (in mm) from the apex to base with a ruler (Appendix 3.6). In addition, digital images were also taken when possible in order to obtain more accurate sizes using image J digital software (Schneider et al., 2012). Measurements were taken once every seven days for a total of 105 days. Unfortunately, limitations in the field meant that sea surface temperature was not recorded for the duration of the experiments. For this reason, temperature was excluded from all analysis in this study.

We used the Shapiro-Wilk test (Shapiro and Wilk, 1972) to check for normality within our barnacle and percentage cover data. After rejecting the null hypothesis (H₀: normally distributed data) in both experiments we performed the non-parametric Kruskal-Wallis test (Kruskal and Wallis, 1952) to check for significant difference in growth rates between the buoys (barnacle experiment) and percentage cover between the nets (percentage cover experiment). After this we fitted linear and polynomial regression trend lines and selected the best fit according to the
highest adjusted R squared value. All analyses were conducted in the statistical programming language R vs 3.4.2 (R Core Team, 2018).

3.3.3 Ghost net data

Drifting ghost nets were collected opportunistically by biologists on board a turtle expedition conducted by the Olive Ridley Project on two separate occasions; November 2017 and February 2019. In 2017 independent nets \((n = 5)\) were immediately stored in 2.5% glutaraldehyde, while due to limitations in the field in 2019 samples could not be fixed on site and only nets found with barnacles were recorded \((n = 3)\). We applied the analyses detailed above to estimate drift times of these nets.

Further, stranded ghost nets occurring in Maldivian waters have been recorded by the Olive Ridley Project between 2013 and 2018. In this timeframe 326 nets have been found \((n = 149\) from the NE monsoon and \(n = 177\) from the SW monsoon) with reported times and locations of recovery but not their age. All nets reported were of twisted construction and made from high density polypropylene (HDPP).

3.3.4 Lagrangian modelling

To explore where ghost nets found within the Maldives may have come from, we used the final net location to backtrack possible origins. This was done using the offline Lagrangian particle dispersal model Ichthyop (Lett et al., 2008; http://www.ichthyop.org/). Diffusion was activated following guidelines by Peliz et al., (2017). Advection was accounted for using 2D hydrodynamic data from Ocean Surface Current Analysis Real-time (OSCAR, Lagerloef et al., 1999; http://www.oscar.noaa.gov). OSCAR is a global current product derived from remote-sensing data and provided on a 1/3° grid with a 5-day resolution. It was evaluated in the tropical Indian Ocean by Sikhakolli et al. (2013).
We initially released 1,000 ‘particles’ at the same time and location as each of the 326 ghost nets found (326,000 particles in total). For the eight age estimated nets we released a further 10,000 particles each (80,000 particles in total). For these nets we used the estimated drift times to set the duration of the simulated backwards drift. For the 326 unaged nets (i.e. those without any prior knowledge of their drift times) we ran the models backwards in time for 10, 30, 60, 90 and 120 days. Plots were generated to illustrate the number of ‘particles’ per 1/4° squares for both ghost nets (n = 326) and ghost net samples (n = 8).

### 3.3.5 Fishery interaction

Data were utilised on the spatial distribution of operating fisheries reported to the Indian Ocean Tuna Commission (IOTC) at a 1° x 1° grid cell resolution to overlay fishing grounds onto our simulations of net origin. To do this, the grid codes from the Coordinating Working Party (CWP) of the Food and Agriculture Organisation (FAO) were converted into latitude and longitude coordinates by finding the centroid of each statistical grid. These were then layered onto the ‘particle’ distribution plots in QGIS (V. 3.4) to highlight fishing activity.

Data and analyses (barnacle growth, percentage cover and ghost net location) was uploaded to the Olive Ridley Project website and is available for download at [https://oliveridleyproject.org/download-minimum-drift-time-data](https://oliveridleyproject.org/download-minimum-drift-time-data).

### 3.4 Results

#### 3.4.1 Experimental assessment of bioaccumulation on floating nets and buoys

Fifteen morphologically distinct diatoms were identified attached to the experimentally deployed nets at Baa Atoll, in the Maldives. These included two asymmetrical biraphid species
from the genus Amphora (Figure 3.1a, b), six monoraphid species (Anorthoneis eurystoma, two Cocconeis sp., Achnanthes pseudobliqua, Achnanthes sp., and Fragilariopsis rhombica, Figure 1c-h). Nitzschia longissima (Figure 3.1i) was also found, along with three symmetrical biraphid species (Gyrosigma tenuissimum, Parlibellus delognei, and Delphineis sp, Figure 3.1j-l), a Hyalosira sp, (Figure 3.1m), a Licmophora sp., (Figure 3.1n) and Gomphonemopsis exigua (Figure 3.1o). Many diatoms were lying flat on the nets (i.e. valve or girdle face in contact with the net, both adnate and motile diatoms). However, some were observed to be erect and attached by peduncle or stalks. The genus Amphora was by far the most frequently recorded (65.6%), followed by the two Cocconeis spp. (8.9%), the Licmophora sp. (6.9%), the Hyalosira sp. (4.1%) and Anorthoneis eurystoma (2.9%). The remaining observed organisms (Achnanthes pseudobliqua, Achnanthes sp. Fragilariopsis rhombica, Nitzschia longissima, Gyrosigma tenuissimum, Parlibellus delognei, Delphineis sp and Gomphonemopsis exigua) accounted for less than 1% of total abundance. It should be noted that these may be an underestimation of the true abundance of diatoms species present as some were positioned on the net showing only their girdle so could not be identified from this orientation (11.5%).
Figure 3.1 Diatoms found attached to the surface of fishing nets, a: Amphora sp. A, b: Amphora sp. B, c: Anorthoneis eurystoma, d: Cocconeis sp. A, e: Cocconeis sp. B, f: Achnanthes pseudobliqua, g: Achnanthes sp.; h: Fragilariopsis rhombica, i: Nitzschia longissimi, j: Gyrosigma tenuissimum, k: Partibellus delognei, l: Delpheeis sp., m: Hyalosira sp., n: Licmophora sp., o: Gomphonemopsis exigua.
Unsurprisingly a large number of bacteria (Cocci shaped) appear on nets in the Maldives within 4 days and rapidly begin to colonise the surface; by number of individuals they are the most abundant fouling organisms (Figure 3.2a). We also observed something encrusting at the same time, but this was not discernible under the scanning electron microscope. Other organisms including amoeboid protists (Foraminifera) such as an *Elphidium* sp. (Figure 3.2b) and a *Foraminifera* sp. (Figure 3.2c) which started to colonise after 42 days and would likely be actively feeding on the diatoms. There were also several unidentified invertebrates, which were observed after 42 days. These included an isopod (Figure 3.2d), an unknown invertebrate (Figure 3.2e) and a bivalve (Figure 3.2f).
Figure 3.2 Rounded cells, Foraminifera and unknown crustaceans and invertebrates. a: bacteria (Cocci); b: *Elphidium* sp., c: *Foraminifera* sp., d: unidentified *isopod* sp., e: unknown invertebrate; f: unknown bivalve.

After 56 days, each net was densely covered in unidentifiable fouling organisms or attached detritus, effectively obstructing the view of any diatoms if indeed present. Therefore, we
truncated diversity analyses for the first 56 days only, and it is suggested that this method for aging nets would only be suitable for the first couple of months at best. Overall, species richness and abundance steadily increased for the first 10 days, reflecting the fast colonisation of a newly introduced ecological niche (Figure 3.3a, b, Appendix 3.7). In our study, early dominance from an *Amphora* spp. was evident (Figure 3.1a, Appendix 3.7). Interestingly, after day 14, diatom diversity decreased (Figure 3.3c, d), but certain species such as *Amphora* sp. (B) and *Licmophora* sp. occurred at this time and were dominant (Appendix 3.7, Figure 3.1b, n). The observed decline in diatom diversity is also reflected in a decrease in evenness (Figure 3.3e). Although not included in the diversity analyses, the heatmap (Appendix 3.7) also highlights that after 14 days, round shaped (*Cocci*) bacteria (Figure 3.2a) peak in abundance and then become difficult to record as percentage cover of biofouling communities increases. Similarly, *Foraminifera* (Figure 3.2b, c) and various unidentified invertebrates (Figure 3.2d-f) first start appearing around 42 days and then dominate as percentage cover of biofouling communities increases, again making it difficult to record the presence and indeed attachment of diatoms to the nets.
Figure 3.3 Mapping variations over time against species richness (a), total abundance (b), Shannon-Wiener diversity (c), Simpson index (d), Pielou index (e), percentage cover of biofouling communities (f) and average capitulum size (mm) of *Lepas anatifera* (g). Error bars equate to standard deviations.
Broadly, the diversity indices illustrate a stochastic relationship between the replicates associated with this study. In contrast, percentage cover of biofouling communities and average capitulum size of the pelagic gooseneck barnacle, *L. anatifera* (Figure 3.3f, g), shows less variation between replicates. Up to 60 days (when percentage cover reaches near 100%), a positive linear relationship occurred (R² = 0.99) (Figure 3.4a). Fitted linear regression models show that the expected (predicted) values match closely with actual values with small confidence intervals (Figure 3.4b).

![Graphs](image)

**Figure 3.4** (a) Biofouling communities can be used to assess age of ghost gear. Linear relationship between number of days and percentage cover of biofouling communities. 100% cover is reached at 56 days; therefore, here we only show the trend from day 0 to this time point. (b) shows the expected value (blue line) and the confidence interval for the expected value represented as the grey band. Partial residuals are shown as dark grey dots. (c)
Polynomial 4\textsuperscript{th} order trend line relationship between number of days and capitulum length (mm) of \textit{Lepas anatifera}. (d) shows the expected value (blue line) and the confidence interval for the expected value represented as the grey band. Partial residuals are shown as dark grey dots.

\textit{L. anatifera} started to appear on the buoys between seven and 14 days after deployment. The largest individual barnacle measured 35 mm in capitulum length (maximum average 27.9 mm) at the end of the experiment (105 days). Growth appeared to slow down as barnacle shell size approached the maximum average size (27.9 mm). A clear sigmoidal curve was observed for the first 105 days when an average of all barnacle sizes was taken for each time interval. A polynomial 4\textsuperscript{th} order trend line (Figure 3.4c) was the best fit for average \textit{L. anatifera} capitulum size ($R^2 = 0.99$). The expected values match closely with actual values and partial residuals show little variance (Figure 3.4d).

3.4.2 Ghost net age estimates and their putative origin

We analysed percentage cover of biofouling organisms and capitulum length of gooseneck barnacles to estimate minimum drift times of eight ghost nets found in the Maldives (Table 3.1). We applied these two methods as they illustrate clear trends and had little variance between replicates in the controlled experiments. We were able to confirm the species of barnacle growing on each net through genetic analyses and all were \textit{L. anatifera}. Although we did not explore diatom diversity, we did observe large clusters of \textit{Amphora sp.} (identified as the same in Figure 3.1b) present on net three, giving an independent indication that this net had been drifting for at least 28 days as inferred by our heatmap and diversity analyses. However, we observed no invertebrates present on any of our ghost nets which contradicts the heatmap (Appendix 3.7). Following this methodology, we expected that ghost net five would have had invertebrates present, which it did not. This further highlight the variation in organism
succession and the unreliability of using diversity indices or key taxa as markers to estimate age alone.

**Table 3.1** Drift time estimates of eight ghost nets found in the Maldives during the south west monsoon (SW, n = 5) and north east monsoon (NE, n = 3). Estimation were found by either measuring the capitulum length of the barnacle *Lepas anatifera* or by analysing the percentage cover of biofouling communities in image J. NA represents no data taken due to limitations in the field.

<table>
<thead>
<tr>
<th>Net ID</th>
<th>Monsoon found</th>
<th>Capitulum length (mm)</th>
<th>Percentage biofouling cover (%)</th>
<th>Estimated drift time (Days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>SW</td>
<td>NA</td>
<td>51</td>
<td>28 (±2)</td>
</tr>
<tr>
<td>2</td>
<td>SW</td>
<td>NA</td>
<td>43</td>
<td>24 (±1.5)</td>
</tr>
<tr>
<td>3</td>
<td>SW</td>
<td>NA</td>
<td>72</td>
<td>40 (±2.5)</td>
</tr>
<tr>
<td>4</td>
<td>SW</td>
<td>NA</td>
<td>16.6</td>
<td>7.5 (±1.5)</td>
</tr>
<tr>
<td>5</td>
<td>SW</td>
<td>NA</td>
<td>89</td>
<td>48 (±3)</td>
</tr>
<tr>
<td>6</td>
<td>NE</td>
<td>5</td>
<td>NA</td>
<td>24 (±2)</td>
</tr>
<tr>
<td>7</td>
<td>NE</td>
<td>26</td>
<td>NA</td>
<td>101 (±10)</td>
</tr>
<tr>
<td>8</td>
<td>NE</td>
<td>18</td>
<td>NA</td>
<td>54 (±2)</td>
</tr>
</tbody>
</table>

Particles were released at each of the eight ghost nets locations and backtracked according to age estimates above (Table 3.1, Appendix 3.8). Simulations for nets one, four (Figure 3.5a) and two (Figure 3.5b) suggest that they originated from inside the EEZ of the Maldives. In contrast nets seven and eight (Table 3.1, Appendix 3.8) showed a comparatively wide dispersal of particles (Figure 3.5b, c) with some drifting close to shorelines of Sri Lanka.
Figure 3.5 Number of particles per 1/4° squares backtracked from each of the eight-net location and grouped to avoid overlapping (a) nets 5, 1, 4, and 6; (b) nets 3, 2, and 8; (c) net 7. Drift duration was set at the estimated drift times calculated for each net. Red areas indicate higher particle density. Maldivian exclusive economic zone (EEZ) is shown as black dotted line.

3.4.3 Putative origin for ghost nets without drift time estimates

At first look, simulations revealed a level of uncertainty with regards to a putative origin for the 326 ghost nets without drift time estimates. This is particularly true for long drift durations such as 120 days in this study. When modelling with the smallest drift time (10 days here), the simulated particles did not travel far outside the Exclusive Economic Zone (EEZ) of the Maldives as indicated by high densities found well within the EEZ (Figure 3.6a-b). The only
fisheries operating in this area (that are reporting to the IOTC) are the Maldivian bait net fisheries (Figure 3.6a-b). At 30 days of drift or more (Figure 3.6c-j) the most likely origins fall outside of this zone and are therefore likely to be coming from other fisheries and EEZs from other countries. The results of the models obviously vary strongly depending on the season, indicating likely hot spots of net origin.
Figure 3.6. Number of particles per 1/4° squares backtracked from the location and time of the 326 ghost nets collected during the NE (n = 149) monsoon (left) and SW (n = 177) monsoon (right). As there was no drift time estimates for these nets drift periods of 10 (a-b), 30 (c-d), 60 (e-f), 90 (g-h) and 120 days (i-j) were used. Red areas indicate higher particle density. Exclusive economic zones (EEZ) are shown as black dotted line for each surrounding country. Maldivian bait fishery area of operation highlighted as white horizontal lines (a-b).

For the first 90 days during the NE monsoon, the model indicated that particles typically drift westerly. Numerous clusters accumulated well within the EEZ of the southern tip of India, Sri Lanka and the Maldives, very close to shore (Figure 3.6a, c, e, g). After 90 days (Figure 3.6i) it became increasingly difficult to determine a putative origin as particles were more dispersed. However, clusters continued to accumulate north of the Maldives within the EEZ of western India and to the east in Sri Lanka and eastern India. Interestingly, particles spread as far as Somalia, Yemen and Indonesia (Figure 3.6g, i) but in lower densities. Comparatively, during the SW monsoon, particle clusters were much more defined, and a putative origin was more prominent in the open ocean of the Arabian Sea (Figure 3.6b, f, h, j). Further, particles aggregated in higher densities when compared to the NE monsoon within the EEZ of Yemen, Oman, western India and western Sri Lanka (Figure 3.6j).

3.4.4 Possible fisheries responsible for ghost nets in the Maldives

For drift times longer than 10 days our simulations suggest that purse seine fisheries (Korea, Mauritius, Philippines, Spain, France and Seychelles) and gill nets from Sri Lanka are ‘high risk’ fisheries (with regard to possible source of lost nets). As these fisheries are active in areas where dense particle clusters occur, particularly at 90 days of drift across both seasons (Figure 3.7a-d). In comparison, ring nets from Sri Lanka appear to be only a seasonal threat (Figure
3.7e-f). However, the ‘level’ of threat from each country appears to vary depending on drift times. For example, nets that have been drifting for 60 days or less during the NE monsoon show a clear overlap in high density areas (red areas) where Sri Lankan purse seine fisheries operate. However, after this time, the dense clusters overlapped more with purse seine fisheries from flags of the EU (Spain and France), particularly after 90 days adrift (Figure 3.7a-b, Appendix 2.8). As drift time increases beyond 90 days to 120 days, particles become more dispersed, making it much more difficult to assign high risk fisheries in the area. Comparatively the SW monsoon simulations provide a clearer putative origin for all drift times analysed in this study. Here the dense clusters of particles push north into the Arabian Sea as drift time increases. For the first 30 days the only major concern is from gill nets operated by Sri Lanka. At 60 days purse seiners from Spain and the Seychelles begin to overlap with these areas of high density and by 120 days purse seine fisheries from Mauritius, Korea, and Seychelles join the ranks of Spain and France (Appendix 3.9).

Although purse seine nets appear to be problematic across both seasons (Figure 3.7a, b), there are temporal changes that influence the risk that specific countries pose. Here, the Sri Lankan purse seine fisheries pose significant risks at all drift times during the NE monsoon, but this is reduced during the SW monsoon. Comparatively, when drift times are more than 60 days during the NE monsoon, purse seine fisheries from France and Spain are the only additional high-risk fisheries analysed in this study during this period. Interestingly, if a net is drifting for only 30 days during the SW monsoon, it is unlikely to be originating from these fisheries. However, if the nets drift for longer periods, fisheries associated with Spain and the Seychelles increase in their likelihood of being the origin of the nets. Korea, Mauritius and France also ‘come into play’ as likely sources when the drift is past 90 days (Appendix 3.10).

It should be noted that Japanese purse seine vessels are also shown to be fishing in areas where high particle density occurs during the NE monsoon. However, a large number of Japanese
coordinates appeared on land when we mapped them. This is obviously an error on the reporting and therefore based on this level of uncertainty we had to exclude Japanese purse seine fisheries from our analyses.
**Figure 3.7** Number of particles per 1/4° squares backtracked from the collected ghost net locations after 90 days of drift during the NE monsoon (left) and SW monsoon (right). Red areas indicate higher particle density. Maldivian exclusive economic zone (EEZ) is shown as black dotted line. Spatial distribution of purse seine fisheries (shown in white shaded area) operating from Sri Lanka, Philippine, Korea, Spain, France, Mauritius and the Seychelles (a-b), Ring net fisheries from Sri Lanka (c-d) and Gill net fisheries from Sri Lanka (e-f).

### 3.5 Discussion

The aging of abandoned, lost or discarded fishing gear (ALDFG), more specifically ‘ghost nets’, is possible, at least to some degree. Percentage cover of biofouling communities and the capitulum size of the goosenecked barnacle, *Lepas anatifera* appeared as the most reliable methods in order to determine minimum drift times due to the low variability between replicates and clear trends in overall measurement. Succession of diatom communities and arrival of rare and macrofouling organisms may also provide additional information that compliments such estimations. The Lagrangian particle dispersal simulation shows the possibility of wide spatial origin of particles (i.e., ghost nets here), arriving in the Maldivian waters yet defines likely hotspots where a putative origin of a given net came from. Overlapping spatial distribution of fisheries onto these simulations allows the identification of fisheries that are most likely to contribute to the ghost nets found in the Maldives archipelago.

**3.5.1 Biofouling communities and ghost gear ageing**

Fishing nets provide a novel and niche habitat for a wide diversity of fouling organisms (Reisser et al., 2014, Fazey et al., 2016, Kooi et al., 2017). Here, we illustrate that a relatively
high diversity of diatoms arrives in the first few days. This is followed by a number of other unidentified organisms including bacteria and invertebrates. Collectively, this is known as the ‘plastisphere’ (Zettler et al., 2013). It is reasonable to assume that this plastisphere may be the initial driver that attracts larger predators such as sea turtles to ghost nets in search of food. Interestingly, we found that bacteria quickly colonise the plastic surface and eventually flourish to become the most abundant organisms. It has previously been shown that these bacteria impact the surface floating plastic, forming pits and groves as a result of biodegradation (Artham et al., 2009, Reisser et al., 2014). This may mean that the bacteria are directly influencing the breakdown of ghost nets.

Percentage cover of biofouling organisms on the experimental nets show a characteristic sigmoidal curve (Figure 3.3f), in a similar pattern to the biofouling which occurs on marine glass samples in Europe (Lehaitre et al., 2008). However, percentage cover does show slight differences between the samples from day 14 to 42. This could be explained, in part by the variation in current, nutrient availability and sea surface temperature which were not recorded in this study. Moreover, our largest barnacle found on the ghost nets (27.9 mm) was of a similar size to that reported previously in temperate waters (25 mm, Evans, 1958). Further, our age estimates and those from this latter paper were similar (101 and 107 days respectively). These latter studies were undertaken in countries other than the Maldives (and the wider Indo-Pacific) indicating that our tools for estimating drift time may well have global reach.

Aging drift times had the highest confidence up to the first 100 days in the water. Percentage cover of biofouling communities become largely unusable after 56 days. However, capitulum size of *L. anatifera* tracked well up to 105 days and possibly expands past this time point as the barnacles in this study had not reached their known maximum growth size (De Wolf, 2008). Capitulum size in excess of 50 mm have been reported for this species (Magni et al., 2015), therefore it is unlikely that maximum size was reached in this study. Moreover Magni et al.
(2008) highlighted that barnacle growth rates may depend on temperature. Therefore, growth rates seen in the Maldives may be different to growth rates found in temperate environments. It is however important to recognise (as stated previously), that surface roughness and topography play important factors in bioaccumulation and growth rates (Scheuerman et al., 1998). Twisted nets are typical of net types found in the Maldives (Stelfox et al., 2015, Stelfox et al., 2019). Future analyses should focus on rates of growth on monofilament and braided nets to compare similarities and differences in bioaccumulation rates and diversity. Additionally, minimum drift time analyses are based on growth rates in coastal environments on fixed objects in tropical waters. This was undertaken for obvious practical and ethical reasons. That said, it should be noted that free-floating ghost nets may well be subject to spatial fluctuations in environmental conditions that may impact biofouling organisms and their succession (Sudhakar et al., 2007).

4.5.2 Lagrangian simulations

Including diffusion in Lagrangian simulations, backwards in time, is a valuable approach to obtain probability distributions of past positions (Batchelder et al., 2006). At least in the context of either limited spatial (here 1/3°) or temporal (here 5 days) resolutions of the forcing current product (here OSCAR). Despite their coarse resolution, current products based on remote sensing data, like OSCAR, have been shown to simulate surface drift trajectories satisfactorily (Hart-Davis et al., 2018, using GlobCurrent) with similar (or even higher) accuracies than higher-resolution oceanographic model outputs (Scott et al., 2012, using Surcouf). Using OSCAR, Davies et al., (2017) showed that simulated trajectories of fish aggregating devices (FADs) were generally consistent with observations. However, in the Indian Ocean OSCAR velocities were shown to be generally lower than velocities derived from oceanographic drift trajectories (Imzilen et al., 2019). That said, the zonal component of OSCAR velocities appears to be more accurate than the meridional component (Sikhakoli et al., 2013).
4.5.3 Fishery interaction

The youngest ghost net analysed in this study (estimated at drifting for only 7.5 days) along with nets one and two (28 and 24 days respectively), suggests an original source origin from inside the EEZ of the Maldives. However, the relatively large mesh size associated with these nets is untypical of those usually used as bait nets in this area, suggesting the possibility of illegal, unreported, and/or unregulated fishing practices taking place within the Maldives. Comparatively, ghost net seven (Figure 3.5c), found during the NE monsoon, was the longest drifter at around 101 days. Multiple clusters were simulated overlapping a wide variety of fisheries including gill and ring nets from Sri Lanka and purse seine fisheries from Spain, France, Seychelles and Korea.

Fisheries operating in the area of study are widespread (IOTC 2018) and those labelled as ‘high-risk’ fisheries (with regard to likely contributors of the majority of nets) fluctuate depending on spatial distribution, season and estimated drift times. However, gill nets from Sri Lanka appear to be high risk fisheries regardless of season at all drift times longer than 30 days. Additionally, the wide dispersal of particles (nets) for the longer modelled drift times (approaching 120 days, Figure 3.6), coupled with the invasion of multiple EEZs particularly close to shorelines of Sri Lanka and India (Figure 3.6) suggest that an unconfirmed proportion of ghost nets entering the Maldives could be from a variety of small scale artisanal fisheries. This is further supported by observations of fragments of ghost nets drifting into the Maldives with Indian markings on floatation devices (Stelfox et al., 2015). Evidence to support the modelling and implicating purse seine fisheries in the origin of a proportion of nets found floating in the Maldives comes from the stranding of FADs (Stelfox et al., 2015, Stelfox et al., 2019). Identifying ghost nets to a specific fishery and/or location can, however, be challenging, as more often than not the gear is unmarked (Stelfox et al., 2015). In the Maldives it is thought that many of the ghost nets that drift into the EEZ originate from neighbouring countries.
(Stelfox et al., 2015), and our Lagrangian simulations support this hypothesis. However, spatial
distribution data from major fisheries such as India, Pakistan, Oman and Yemen and those from
coastal fisheries from all surrounding countries (which dominate the Indian Ocean) are sparse and, in some cases, completely absent in the IOTC database (IOTC 2018). This lack of reporting means that although our analysis is as thorough as possible, we may still be overlooking the risk of the fisheries associated with these countries (where no spatial distribution data is available).

4.5.4 Future recommendations

A lack of data on gear types and spatial distributions of small and large-scale fisheries means it remains difficult to accurately identify responsible fisheries in the Indian Ocean. The IOTC working party on data collection and statistics note this to be the case especially for artisanal fisheries and in particular for gill nets (IOTC 2018b). A cross sectorial collaboration between governments, NGOs, IGOs and the private sector should take precedent, aimed at improving the collection and transparency of such data and share resources where possible. Simulations and age estimates (combined), do however highlight that purse seine fisheries and gill nets are likely key candidates for a major part of the ghost gear found drifting into the EEZ of the Maldives. To minimise damage to sensitive habitats in this country, a recovery project (similar to “FAD watch” set up in the Seychelles; Zudaire et al., 2018) could be initiated throughout the Maldives. Such a project would require the collaboration of Maldivian NGOs, the government and purse seine fisheries from the EU (Spain and France), Mauritius, the Seychelles and Korea. In the Seychelles, FAD watch allows for the recovery of FADs that come within 3-5 nautical miles of selected islands in the Seychelles. Through this project a 20% and 41% reduction in beaching events have been observed in 2016 and 2017, respectively. The IOTC play an important role in ensuring that projects such as “FAD watch” are encouraged amongst its country members and that compliance amongst all country members on reporting
FAD deployment and gear loss should be improved as a matter of urgency. Further the IOTC need to revisit the number of FADs deployed by country members and consider stricter restrictions on the number of FADs deployed by each vessel. This reduction will not only help reduce gear loss but also reduce bycatch associated with purse seine fisheries. Further research by the International Seafood Sustainability Foundation (ISSF) and other organisations developing gear design to reduce entanglement from FADs should take priority to ensure that any lost gear results in minimal entanglement.

It should also be noted that for the modelling, we assumed ghost nets behave like particles and we do not include net geometry and/or vertical profile or the impact of stokes drift into account (Dobler et al., 2019). Future analyses of ghost net drifts should focus on how bioaccumulation may impact floating times and vertical profile and how ghost net geometry may influence drifting trajectories. Although diversity indices were not effective in estimating age in this study, different species of diatoms and other macrofoulers can provide additional tools to help estimate drift times. Categorising bioaccumulation composition for different regions may help identify region specific organisms that could give additional clues towards drift trajectories and ghost gear origins. Moreover, future research on biofouling rates in the region should focus on the impact of environmental conditions such as sea surface temperature, nutrient availability and the impact of currents on these rates. Finally, we only report diatoms that could be visually identified, future analyses of fouling communities should focus on utilising molecular tools to increase the known diversity at any given time point.

Although analysing trajectories of satellite tagged turtles is beyond the scope of this study. It is worth highlighting that future research should aim to combine species distribution and migration data, in this case for olive ridleys, with spatial data on fisheries to uncover overlaps and interactions. For example, pelagic shark species were tracked in the north Atlantic uncovering predictable hotspot areas of high use in areas where Portuguese and Spanish
longliners operate (Queiroz et al., 2016). We could implement a similar methodology to uncover ghost gear and olive ridley interactions in the Indian Ocean and possibly use this as an additional tool to build evidence on the true impact that different fisheries have on sea turtles in the region.

To conclude, here in this study we illustrate that percentage biofouling cover and capitulum length of the gooseneck barnacle, *Lepas anatifera* can be used to infer minimum drift times of ghost gear. We show that nets in the Maldives have a minimum drift times from as little as 7.5 days to over 101. Our back-track modelling suggests that nets with shorter drift times (<30 days) likely originate from illegal, unreported and unregulated fishing which is occurring within the Maldives EEZ. Whilst those drifting for longer than 30 days appear to be originating from purse seine and gill nets fisheries (from a number of countries), along with smaller scale artisanal fisheries in areas such as Sri Lanka.

### 3.6 Acknowledgements

We are grateful to all the volunteers of the Olive Ridley Project for collecting and reporting ghost gear in the Maldives and wider Indian Ocean. We thank Jenni Choma, Sonia Valladares, Claire Nerissa, Claire Petros, Deborah Burn and Josie Chandler for assisting with experimental deployment and data collection. We would also like to extend our gratitude to Coco Palm Dhuni Kolhu Resort, Baa atoll and Bodu Hithi resort, North Male atoll for allowing net experiments in their lagoons. Thank you to the team at SureScreen Scientifics, particularly Troy Whyte and Arthur Green for the use of the SEM. Thank you to Gareware Wall Ropes Ltd. for supplying new nets for bioaccumulation analyses.
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https://doi.org/10.1007/s12040-012-0258-7


Appendix

Appendix 3.1 Map showing the geography of the Maldives and Northern Indian Ocean (5 x 5° grid resolution). The Maldives is enclosed along its northern border by Indian, Pakistan, Iran and Oman.
Appendix 3.2  Map showing Maldivian archipelago at 2 x 2° grid resolution. Green diamond represents the location of the diatom diversity and biofouling percentage cover experiment in Baa atoll. Red diamond represents the location of the growth rate of gooseneck barnacles (Lepas anatifera) experiment situated in North Male atoll.
Appendix 3.3 Map showing the Maldivian archipelago at 2 x 2° grid resolution. (TOP Image) GPS location of ghost nets (blue dots) collected during the north east monsoon (n = 149). (MIDDLE image) GPS location of ghost nets (red dots) collected during the south west monsoon (n = 177). e) (BOTTOM image) GPS location of the 8 ghost nets (orange dots) collected and analysed for drift time estimates.

Appendix 3.4 Illustration of the experimental set up of floating nets (a) illustrates the side profile of the fixed rope between the two floating buoys (red ball) in 18m of seawater. (b) Illustrates an aerial view of the same experiment showing the three net replicates attached to the fixed rope with antifouling zip ties. All nets are positioned with enough distance between them and their own individual plastic float ensuring no contact was possible with the neighbouring net.
Appendix 3.5 Net sample taken for biofouling percentage cover and diversity index analyses. Red area indicates the sample taken from each net on day two, four, six, eight and ten, then every two weeks thereafter up until 112 days. Sample size from knot to base of twine is 3cm.
Appendix 3.6 Capitulum measurement of the gooseneck barnacle *Lepas anatifera*. Red line indicates how the capitulum length was measured including the main body and all plates but excludes the peduncle (i.e. stalk).
Appendix 3.7 Heat map showing Z-score hierarchical clustering based on Euclidean distance measure. Each row represents biota species and columns represent time elapsed in days. The z scores colour scale shows the standard deviation from the mean of each number of individuals within each row (species), whereby the lighter colour (towards white) highlights low expression and the darker the colour (red) the higher the expression. Species identification can be found in Figures 4 and 5, *Amphora sp.* (A) (Fig 4a), *Amphora sp.* (B) (Fig. 4b), *Cocconeis sp.* (A) (Fig. 4d), and *Cocconeis sp.* (B) (Fig. 4e).
Appendix 3.8 Eight ghost nets age estimates using percentage cover of biofouling communities (left) and *Lepas anatifera* capitulum length (mm). All ghost nets found in the Maldivian Archipelago.
Appendix 3.9 Raster plots showing the number of particles per $1/4\degree$ after 120 days during the NE monsoon (a-d) and SW monsoon (e-j). Red areas indicate higher particle density. Maldivian exclusive economic zone (EEZ) is shown as black dotted line. Spatial distribution of gill (a), ring (b) and purse seine nets from Sri Lanka (c) and purse seine nets from the Philippines (d), France (e), Korea (f), Mauritius (g), Seychelles (h), Spain (i) and gill nets from Sri Lanka (j).

Appendix 3.10 Raster plots showing the number of particles per $1/4\degree$ after 90 days during the NE monsoon at 90 days drift times. Red areas indicate higher particle density. Maldivian exclusive economic zone (EEZ) is shown as black dotted line. Spatial distribution of purse seine fisheries operating from Spain (a) and France (b).
Chapter 4. Tracing the origin of olive ridley turtles entangled in ghost nets in the Maldives: a phylogeographic assessment of populations at risk

4.1 Abstract

Abandoned, lost or discarded fishing nets, (ghost nets) represent a major threat to marine vertebrates. However, thorough assessments of their impact on threatened species are largely missing. In the Maldives, olive ridley sea turtles (*Lepidochelys olivacea*) are frequently caught in ghost nets however the archipelago does not support a significant nesting population. Our aim in this study was to determine the origin of olive ridleys entangled in ghost nets found in the Maldives and evaluate potential impacts on respective source populations.

Based on a citizen science and conservation program, we recorded 132 olive ridley turtles entangled in ghost nets in just one year. Genetic analyses (mtDNA) of entangled individuals and of potential source populations revealed that most captured olive ridleys originated from Sri Lanka and eastern India. Oman could be excluded as source population, even during the prevalence of the south west monsoon. Based on our results and already available published literature, we were able to estimate that the recorded ghost net entanglements accounted for a relatively small amount (0.48%) of the eastern Indian population. However, the entangled turtles accounted for a much larger percentage (41%) of the Sri Lankan populations. However, it should be noted that our estimates of population-level mortality are linked to substantial uncertainty due to the lack of reliable information on population dynamics. Consequently, any precautionary protection measures applied should be complemented with improved quantification of turtle recruitment and life-stage specific mortalities.

4.2 Introduction

Over 1 million species are globally threatened with extinction, a result largely attributed to changes in land and sea use and direct exploitation of organisms (UN 2019). One group under
severe threat includes marine reptiles (Gibbons et al., 2000, Fuentes et al., 2012). Some of the main threats for marine reptiles and turtles, in particular, are incidental capture or bycatch during active fishing practices (Lewison and Crowder, 2007, Cuevas et al., 2018, Alfaro-Shigueto et al., 2018), unregulated coastal development (Harewood and Horrocks 2008; Mazaris et al., 2009, Dimitriadis et al., 2018), predation from humans (both on nests and adults; Allen et al., 2001, Mancini et al., 2001, Engeman et al., 2006, Koch et al., 2006, García et al., 2006) and entanglement in abandoned, lost or discarded fishing gear (ALDFG) (Jensen et al., 2013, Wilcox et al., 2013, Stelfox et al., 2016, Duncan et al., 2017). The quantitative evaluation of these threats must be a focal point of research in order to support the development of conservation strategies and prevent further local and global species extinctions.

Despite the widely acknowledged importance of ghost fishing (Wilcox et al., 2016, Stelfox et al., 2019), its effect on sea turtles and other marine vertebrate populations is substantially understudied (Nelms et al., 2015, Stelfox et al., 2016). Current knowledge gaps may be a direct result of methodological difficulties in quantifying ghost net entanglements. Ghost nets can travel long distances (Wilcox et al., 2013), even across entire oceans (Sayer and Williams, 2015). However, travel paths of ghost nets represent a major uncertainty. Moreover, the often-stochastic distribution of ghost nets substantially complicates the quantification of these nets and its impact on marine life. An accurate impact assessment of ghost nets requires continuous screening over different seasons coupled with quantitative records of turtle entanglement events. Further, genetic analyses of entangled individuals would be ideally acquired in order to identify their potential source populations (population defined in a conservation context, see Moritz, 1994). In the Maldives, olive ridleys appear to be particularly susceptible to entanglement in ghost nets and have been recorded in high numbers throughout the year (Stelfox et al., 2015). While other turtle species are reported to be more threatened by entanglement in other areas of the world, the high frequency of olive ridley turtle entanglements
in the Maldives (Stelfox et al., 2019) provide an opportunity to assess the impact of ghost nets on the viability of turtle populations. We also hypothesise that since records of nesting olive ridleys in the Maldives are extremely rare (Stelfox pers. comm), the majority of entanglements are likely to be affecting non-local source populations. Determining the origin of the turtles entangled therefore requires data on their genetics. Importantly, one of the largest gatherings of nesting olive ridleys annually occurs a little north east of the Maldives. These *arribadas* (Spanish for arrival; Shanker et al., 2004) have been well documented along several beaches off the east coast of India. The genetic structure of these eastern Indian populations has also been relatively well investigated, similar to the populations of Australia and Sri Lanka (Bowen et al., 1997, Shanker et al., 2004, Jensen et al., 2013). In contrast, however little is known about haplotype composition and frequency of nesting populations in other areas of the Indian Ocean such as Oman and on the southern and eastern African coasts (Rees et al., 2012). This data gap could be especially critical as seasonal switches in ocean currents occur between the south west (SW) and north east (NE) monsoon is likely to be important with regard to untangling where the ghost net turtles in the Maldives are coming from. Further, the importance of so called ‘orphan haplotypes’ i.e. turtles with a genetic structure not linked to a known nesting population has been shown in a previous study (Jensen et al., 2013). Indeed, in this study focused around northern Australia (Jensen et al., 2013), 45% of the turtles assessed belonged to this category. Knowledge of such orphans obviously complicates the design and implementation of management plans of these turtles and the threats they face but is none the less important if we are to effectively limit the effect ghost nets has on this species.

Mitochondrial DNA (mtDNA) is often used to uncover ancestral lineages to determine evolutionary history. For example, a global analysis of olive ridley sea turtle mtDNA suggests that the Atlantic and east Pacific populations were recently colonised by the Indo-West Pacific region (Bowen et al., 1997). Another useful application of mtDNA is identifying genetic
structure to determine conservation importance, connectivity and define management units within species (Phillott, & Gamage, 2014). Understanding genetic structure helps identify unknown individuals from a mixed population (mixed stock) (Bolten, et al., 1998, Bolker et al., 2007, Bjorndal et al., 2008, Proietti et al., 2009, LaCasella et al., 2013), particularly useful for stranding and bycatch analyses. As mentioned above, the genetic structure of olive ridley sea turtles in the Indian Ocean are relatively well documented along the east coast of India (Shanker et al., 2004), Sri Lanka (Bowen et al., 1997) and northern Australia (Jensen et al., 2013). However, many data gaps still exist, particularly in the northern Indian Ocean at nesting sites in Oman, eastern Africa, Bangladesh and Pakistan (Phillott & Gamage, 2014). Previous resolution of olive ridley genetic structure in the northern Indian Ocean were based on the 400 - 470bp mtDNA control region (Shanker et al., 2004, Bowen et al., 1997). However more recent analyses of the northern Australian populations were based on the longer 880bp fragment (Jensen et al., 2013). Longer sequences provide improved genetic resolution and it has been suggested that future analyses of genetic stocks should focus on these longer sequences to improve genetic resolution allowing for more effective conservation measures (FitzSimmons and Limpus, 2014).

Our aim, therefore, was to assess the impact ghost nets (recorded in the Maldives) had on known olive ridley nesting populations (i.e. source populations) and determine the origin of the entangled individuals. To achieve this goal, we first built on existing knowledge of phylogenetics of nesting olive ridleys across the Indo Pacific (Kartik et al., 2004, Bowen et al., 2004, Jensen et al., 2013). These were then compared to mitochondrial haplotypes of entangled individuals in the Maldives (i.e. mixed stock analysis). We then attempted to identify if previously reported ‘orphan’ haplotypes (Jensen et al., 2013) originated from our nesting stock and/or were captured in the genetics of the entangled turtles. Finally, we wanted to explore if season (north east (NE) or south west (SW) monsoons) impacted where the turtles were
originating from (Shankar et al., 2002). This was undertaken using a citizen science approach to monitor ghost net catches over a 12-month period across the archipelago. It was hypothesised that those entangled during the NE monsoon would stem from Sri Lanka and eastern Indian genetic stocks. Whilst those entangled during the SE monsoon would originate from turtles nesting in sites such as Oman.

4.3 Materials and Methods

4.3.1 Monitoring of ghost nets in the Maldives

We used a citizen science approach applied by the Olive Ridley Project (ORP registered charity no. 1165905) to document ghost nets drifting into the Maldives over a 12-month period (February 2017 – February 2018). Since 2013, ORP have run scientific workshops throughout the Maldives, explaining how people (citizen scientists) can safely rescue sea turtles and, where possible collect data on ghost net findings and entanglement cases. Participants were also encouraged to propagate their knowledge to other members of their communities, which allowed ORP to capitalise on multiplication effects and increase the network of data collectors. It should be noted however, that the majority of resorts (where the ORP citizen scientists are predominantly based) are situated in only two of the 26 atolls; North Male and Baa atoll. The data provided in this study therefore only reflects a relatively small portion of the Maldivian archipelago and is likely an underrepresentation of the true number of ghost net and entanglement events.

Data on net characteristics (e.g. mesh size, twine diameter and colour), presence or absence of turtles (species verified by the authors through photo identification), GPS coordinates and date
found were recorded via an online portal hosted on the ORP website (https://oliveridleyproject.org/report-a-ghost-net). When entangled turtles were encountered by our citizen scientists, the turtles were sent to the ORP rehabilitation facility in Baa atoll. Once at the centre, small tissue samples (Permit No. EPA/2017/PSR-T02) were collected from the front left flipper by ORP veterinarians. If this flipper was damaged or missing, alternative flippers were used. In some cases, tissue samples were collected in situ by marine biologists (trained in this task during our workshops) before going to the ORP rehabilitation centre. All samples were immediately stored in 100% ethanol and placed in the refrigerator at -4°C. Accompanying each sample was information on the ‘incident’, which included: turtle species, sex (if known), curved carapace length (cm), injuries sustained from entanglement, GPS coordinates of where found and date of rescue.

4.3.2 Genetic characterisation of source populations

Initially, we performed an extensive literature search to identify what previous genetic analysis had been undertaken of olive ridley turtles across the Indian Ocean. First, key word searches on Google Scholar and Science Direct were performed using the search terms “genetics”, “mtDNA”, “haplotypes”, “haplotype network”, “Indian Ocean”, “bycatch”, “population”, “phylogenetics”, “ghost nets” and “ALDFG” in combination with “olive ridley” or “Lepidochelys olivacea” to identify relevant literature. The resulting articles were used as a starting point to identify additional references providing further data. Our search identified only three articles providing haplotype composition and frequency data for olive ridley sea turtles in the Indian Ocean or wider Indo-Pacific region. These included rookeries along the east coast of India (n = 65 turtles, excluding offshore capture; Shanker et al., 2004), Sri Lanka (n = 17 turtles; Bowen et al., 1997) and northern Australia (n = 102 turtles; Jensen et al., 2013) (Figure 4.1). Additional data from Peninsular Malaysia and Andaman and Nicobar Islands were excluded because of either low replicate numbers (≤5) or lack of sequence data (Bowen et al.,
1997, Shanker et al., 2011). All studies were based on sequences of the mtDNA control region, however, studies differed in the length of amplified fragments. For example, the Australian samples were longer (770 bp fragments), whilst the available sequences from turtle populations across eastern India and Sri Lanka were considerably shorter (410 bp or 470 bp).

We complemented the available (previously published) data by analysing additional samples from Oman and eastern India. In Oman, samples from females nesting along a 4 km stretch of beach in Masirah island (n = 33 turtles) were provided to us by the Environment Society of Oman (ESO) (CITES No. 34/2016, 40/2018). These samples were collected during the peak nesting season (February – April) between 2013 and 2017 along with turtle measurements and Photo-ID to ensure repeat nesters were not sampled twice in this study. Samples from nesting females in Rushikulya, India (n = 9 turtles), were collected by K. Shanker to give us the larger 770 bp fragment data for this region (Figure 4.1). Additionally, a small sample of turtles caught as fisheries bycatch (collected by Kelonia) off the coast of Madagascar (reportedly by French longline fisheries) were also included (n = 9), in order to extend our data collection and potentially identify additional haplotypes.
Figure 4.1 Nesting sites (triangles), sampling locations of turtles entangled in ghost nets in the Maldivian Archipelago (circles) and bycatch recorded in coastal waters off Madagascar (diamonds). Samples include rookeries in Oman ($n = 33$; sampled in this study), India ($n = 74$, including 9 sampled in this study), Sri Lanka ($n = 17$), Australia ($n = 102$) as well as ghost net samples from the Maldives ($n = 45$) and bycatch from Madagascar ($n = 9$), both sampled in this study.

All samples were collected as small (2 – 4 mm) tissue biopsies from the flippers as was the case for those entangled in ghost nets. After collection, samples were preserved in 100% molecular grade ethanol and kept at $-4^\circ$C until extraction.

4.3.3 mtDNA extraction and amplification
DNA was extracted from turtle tissue using the DNeasy® Blood & Tissue Kit and following manufactures recommendations. First, DNA quantity and quality were checked by running 1 µl through a spectrophotometer (NanoDrop 2000) before being prepared for amplification. Extracted samples were amplified by polymerase chain reaction (PCR) using the forward primer LTEi9 (5’-AGC GAA TAA TCA AAA GAG AAG G-3’) and reverse H950 (5’-GTC TCG GAT TTA GGG GTT TA-3’) (Abreu-Grobois et al., 2006). These primers target the 880 bp fragment of the mtDNA region. PCRs were run at a 25 µl volume which included, 12.5 µl of PCRBIO Ultra Mix Red (PCRBIO Ultra Polymerase, 6 Mm MgCl₂, 2Mm dNTP), 1 µl of each primer, 9.5 µl of SIGMA H₂O and 1 µl of template DNA. PCRs were performed on a GeneAmp® 9700 PCR system following a denaturation step of 95 ºC for 5 min followed by 45 cycles of denaturation (45 s at 94 ºC), annealing (45 s at 56 ºC), extension (45 s at 72 ºC) and a final extension for 5 min at 72 ºC. Analyses of PCR products were performed by gel electrophoresis and imaged using Thermo Scientific. Unsuccessful PCR amplifications went through a second PCR and if still unsuccessful the original tissue was re-extracted. Sequencing of forward and reverse reactions was carried out by Eurofins Scientific and at the Centre for Cellular and Molecular Platforms (C-CAMP, NCBS), Bangalore, India.

4.3.4 Data analyses

All sequences obtained in this study and from previously published literature were aligned using Clustal W within the Geneious (v11.1.5) software. Newly sequenced samples were manually checked for ambiguity of base-pair identification using an electropherogram and ambiguous sequences from the Maldives (n = 7), Oman (n = 4) and east India (n = 2) were excluded from further analyses.

After quality control, we identified the number of haplotypes in DNAsp V 6.12 (Rozas et al., 2003). A haplotype was defined as a unique sequence differing from other sequences at any
nucleotide site. These differences may be described as one or a combination of nucleotide transitions, transversions or INDELS.

Common indices of biodiversity including haplotype richness and evenness (Pielou, 1966) and both weighted and unweighted phylogenetic diversity (Faith, 1992) were computed in R (v 1.1.3; R Core Team 2019). Rarefaction was performed before analyses on all above-mentioned indices (except haplotype evenness). However, as the population with lowest sequenced individuals (Sri Lanka; \( n = 17 \)) had much fewer individuals than other populations, we excluded Sri Lanka from rarefaction. Hence, comparisons between the diversity of olive ridley populations in Sri Lanka and other populations must be treated with caution as the different sampling efforts could affect the results. For the above, analyses were performed using the shorter 410bp segments in order to maximise the number of available individuals.

We then investigated differences in population structure and composition. We generated two medium joining networks (haplotype networks) using the software NETWORK (v 5.0; Fluxus Technology Ltd.). One was undertaken on the short sequences (410 bp) as above and was constructed to show the relationship between haplotypes from published rookeries and those collected in this study. The second version was run on longer sequences up to 770 bp and was constructed to uncover haplotype divergence from the shorter fragment and to potentially identify the origin of orphan haplotypes collected in ghost nets in Australia (i.e. a haplotype not recorded from any source populations) (Jensen et al., 2013). We followed the software user guidelines and applied a double weighting to characters that had deletions or insertions for both networks.

Moreover, we tested whether different nesting sites showed significant differences in their haplotype composition (defined by the shorter 410 bp fragment lengths) in a Discriminate Analyses of Principal Components (DAPC) following Jombart et al. (2010). In brief,
mismatches in nucleotide bases (A, C, T and G) of aligned mtDNA were vectorised into a
binary matrix (every allele was coded as one variable; 1 s and 0 s indicated presence/absence
of alleles in individual haplotype). Further, we used a Principal Component Analyses (PCA) to
reduce the number of variables (alleles). The PCA allowed us to use 10 principle components
(95% of variance explained) as input for the discriminate analyses (DA), which is limited by
the number of allowed input variables (Jombart et al., 2010). The DA was performed using the
different rookeries (Fig. 1). After the completion of the DA, we used a Permutational
Multivariate Analyses of Variance (PERMANOVA) to test for significant differences between
rookeries and identify genetic structuring between populations. Because multiple pairwise
comparisons were performed, we applied a Holm-correction to adjust p-values (Holm, 1979).
DAPCA and PERMANOVA were implemented in R using the ‘MASS’ package (Ripley et al.,
2013).

Finally, we assessed the contributions of different source populations to entangled turtles found
in Maldivian ghost nets using a ‘mixstock’ analysis (Bolker et al., 2007). Mixstock relies on a
‘winBUGS’-based Bayesian algorithm that uses Monte Carlo Markov Chain (MCMC)
procedures and a hierarchical model structure (Bolker et al., 2007) aimed at estimating the
contribution of different sources to a mixed sample. In order to achieve this, we first divided
our samples into two groups according to season (NE: n = 21, and SW: n = 15). The results of
the two analyses were very similar (Appendix 4.1) therefore we reran our analysis with all
samples pooled (n = 38); this allowed us to include samples that had no date recorded (n = 2).
Again, we used 410 bp fragments to maximise the power of our analysis.

Mixstock provides the option to include the different sizes of source populations into
calculations (Bolker et al., 2007). Therefore, we compiled estimates of population sizes for all
rookeries (Appendix 4.2) and used these estimates as additional input for a second mix-stock
analysis using all ghost net samples.
For each analysis, five chains were run at 20,000 steps, each with 10,000 used as burn in. To ensure correct chain convergence with the posterior probabilities, we used the Gelman-Rubin diagnostic (Gelman and Rubin, 1992) and ensured shrink factors remained below 1.2 (Pella and Masuda, 2001). Individuals with orphan haplotypes (haplotypes only found in ghost nets but in no source population) were removed before the final mixed stock analysis \( (n = 1) \).

### 4.4 Results

Between February 2017 and February 2018, 177 ghost net fragments (incomplete and damaged fishing nets) were recorded (see Stelfox et al., 2019 for more detailed information about net types and responsible fisheries). In these nets, a total of 137 turtles were entangled, including 1 green (curved carapace length not available), 4 hawksbills (curved carapace length range: 30 – 40 cm) and 132 olive ridley turtles (curved carapace length range: 13 - 70 cm). Most net fragments entangled only one turtle; however, 20 net fragments entangled multiple turtles (between two-seven) and four turtles were found dead. Net fragments varied in size from large mono-specific gear (in excess of two tonnage) to small football sized fragments. Due to limitations in the field (e.g. sample transport), immediate release upon rescue by volunteers without collection of tissue, and removal of sequences after quality control, we were only able to attain 38 high quality olive ridley sequences in this study. Entangled olive ridleys were found during both monsoons, NE \( (n = 21) \) and SW \( (n = 15) \) (exact sampling time records missing for two individuals). The olive ridleys which were sequenced \( (n = 38) \) ranged in size from between 13 and 69 cm in curved carapace length with 74% \( (n = 26) \) being classed as 60 cm or smaller, i.e. sub adults or juveniles (Appendix 4.3). Entangled turtles displayed a wide variety of injuries ranging from shallow lacerations around the flippers and neck to deeply embedded entangling...
wounds and complete flipper amputations (Figure 4.2). In some cases, severe dehydration and death were also reported.

Figure 4.2. Minor injuries across the right and left anterior flipper as a result of ghost net entanglement in the Maldives (left), Picture credit: Olive Riley Project. Complete amputation of right flipper as a result of entanglement in ghost nets in the Maldives (right), Photo Credit: Claire Petros.

4.4.1 Diversity and genetic structure of source populations

Four populations (according to nesting country) were assessed in this study. For the shorter sequences (including those truncated from this study), the different populations were characterised by a relatively similar haplotype richness (Figure 4.3a). That is except for Oman, which showed a lower value (two haplotypes compared to four found in Australian, Sri Lankan and east Indian populations). The two haplotypes occurring in Oman showed the same relative abundances to each other, resulting in an evenness of 1 (Figure 4.3b). In comparison, Indian rookeries (although comprised of four haplotypes), were dominated by one Lo44 (Accession # MN342241), which was reflected in a comparatively lower evenness of 0.34 (Figure 4.3b). Interestingly, genetic diversity was lower within Australian rookeries, indicated by low
unweighted as well as weighted faith indices (Figure 4.3c, d). This suggests a close relationship among these Australian haplotypes. While unweighted phylogenetic diversity was similar among all other rookeries, Sri Lanka showed the highest weighted genetic diversity (Figure 4.3D). Despite lower sample size, Sri Lanka was the only rookery excluded from rarefaction due to the lower sampling effort and number of sequences therefore available to us.

**Figure. 4.3.** The genetic diversity of olive ridley turtles (*Lepidochelys olivacea*) at different nesting sites and of individuals entangled in ALDFG in the Maldives. Displayed are (a) haplotype richness, (b) evenness of haplotype frequency (c) unweighted phylogenetic diversity and (d) weighted phylogenetic diversity. Phylogenetic diversities were calculated using the faith indices (Faith, 1992). All data for these analyses were based on 410 bp fragments of mtDNA. Prior to calculations for a, c and d, data has been rarefied. The rookeries from Sri Lanka were excluded from rarefaction (indicated by *) due to lower sample numbers (*n* = 17). Consequently, comparisons to other sites need to be applied with caution.
Our haplotype network (created from the 410 bp fragments – Figure 4.4a), provides a general overview of genetic structuring between nesting sites from Australia \( (n = 102) \), eastern India \( (n = 72) \), Sri Lanka \( (n = 17) \) and Oman \( (n = 29) \). Broadly, this network highlights the separation of two major haplotype groups that are separated by a large genetic distance (7 bp INDEL, one transversion and nine transitions). Within each of the two groupings, a relatively low degree of divergence is observed and haplotypes cluster around the two dominating haplotypes Lo44 (‘K’ in Shanker et al., 2004 and Bowen et al., 1997) and Lo1 (‘J’ in Shanker et al., 2004 and Bowen et al., 1997). Haplotype Lo44 was predominately found in eastern Indian rookeries and the majority of other Indian samples belonged to closely related haplotypes. Lo1 was dominated by Australian samples and again, other Australian samples were attributed to relatively closely related haplotypes (Figure 4.4a). In contrast, samples from Sri Lankan and Omani rookeries contained haplotypes belonging to both Lo1 and Lo44 haplotype clusters. Consequently, turtles from these nesting populations illustrate relatively large genetic distances between the individuals assessed. Likewise, Maldivian ghost nets and French fishery bycatch samples included individuals from both haplotype groups.
Figure 4.4 Olive ridley (*L. olivacea*) haplotype networks based on a medium joining algorithm for the 410bp fragment (a; \(n = 269\)) and 770 bp fragment (b; \(n = 229\)). Circles represent individual haplotypes and circle size reflects the relative abundance of each haplotype across all samples. Colours represent the relative contribution of different nesting populations, ALDFG and bycatch samples to individual haplotypes. Cross lines along connections between
haplotypes represent a nucleotide mutation between haplotypes. Medium vectors (mv) are hypothesised ancestral links or missing rookeries between nodes required to link the shortest connection with maximum parsimony represented here by grey lines. b includes haplotypes found in ALDFG in Australia recorded by Jensen et al., 2013.

We also constructed a second haplotype network based on the larger 770 bp fragment. This was including a lower number of samples but it allowed us to analyse divergence from the shorter fragments (Figure 4.4b). This second network also allowed us to attempt the identification of orphan haplotypes previously identified in a study from northern Australia (Jensen et al., 2013). By using these primers (resulting in the longer bp fragments) we were able to extend sequence length for three existing haplotypes (Lo44 and Lo50 – Accession # MN342242) initially documented from east Indian rookeries by Shanker et al., (2004) as ‘K’ and K4 respectively, and haplotype Lo42 (Accession # MN342240) described by Bowen et al., (1997) as haplotype ‘I’ in Sri Lankan rookeries (Appendix 4.4). The additional genetic resolution (stemming from the larger fragment length analysed) resulted in a subdivision of Lo1 (central among Australian populations) and indicated a few haplotypes (n = 7) which diverged from Lo1 into Lo15 (see Jensen et al., 2013). Similarly, haplotype Lo44 (the primary haplotype associated with Indian rookeries) diverged into haplotype Lo97 (Accession # MN342235 – Appendix 4.5). In total, our study revealed the existence of four ‘new’ haplotypes, one from east Indian rookeries (Lo97), one from the Omani rookery (Lo99 – Accession # MN342236) and two ‘orphans’ which were from entangled turtles collected in the Maldives (Lo98 and Lo100 – Accession # MN342237, MN342238 respectively). Interestingly, over 50% of the Omani turtles analysed were shown to have a unique, previously unidentified haplotype (the Lo99 – see above) which differed from others by only one mutation from the more common Lo44 which itself is shared across the eastern Indian rookeries (Figure 4.4b).

4.4.2 Difference between source populations
Some of the nesting populations (Australia and eastern India) included data from several rookeries. Utilising this (and by conducting a PCA and DA), we assessed whether different rookeries show significant differences in their haplotype composition (Table 4.1). Not surprisingly, we found no significant differences between the eastern Indian rookeries (Table 4.1), a result confirming earlier findings by Shanker et al. (2004). Subsequently east Indian rookeries were therefore able to be pooled as one population and treated as a separate but single genetic stock. Australian rookeries, however, showed significant differences between Flinders beach and Tiwi Island (\( F = 6.022, p = 0.008 \)). This again supported findings by Jensen et al. (2013), who grouped rookeries into two populations, those from the Northern Territory (NTAus), i.e. McCluer group and Tiwi islands, and those from the Cape York Peninsula (CYPAus), i.e. Flinders beach. These two genetic stocks were therefore retained for future analyses (Appendix 4.6). Omani and Sri Lankan populations remained separate from the two Australian and the single east Indian genetic stocks and so were both considered as two separate genetic stocks (source populations). The relationship between geographic distance and genetic distance amongst the four broad nesting populations (Australia, east India, Sri Lanka and Oman) are shown in Appendix 4.7.

Table 4.1 Pairwise comparisons between different rookeries following DAPC analyses. Displayed are F-values (below diagonal) and p-values (above diagonal) of pairwise comparisons between rookeries (FB = Flinders beach, MG = McCluer Group, TI = Tiwi
islands, MAD = Madras, GN = Gahirmatha, DVM = Devi River Mouth, RU = Rushikulya, SL = Sri Lanka). All test was based on the 410 bp sequence fragment of the control region of the mtDNA. Non-significant values are highlighted in bold and holm correction of multiple tests are shown in parenthesis.

<table>
<thead>
<tr>
<th></th>
<th>FB</th>
<th>MG</th>
<th>TI</th>
<th>MAD</th>
<th>GN</th>
<th>DVM</th>
<th>RU</th>
<th>OMAN</th>
<th>SL</th>
</tr>
</thead>
<tbody>
<tr>
<td>FB</td>
<td>0.423(0.423)</td>
<td>0.001(0.008)</td>
<td>0.001(0.008)</td>
<td>0.001(0.008)</td>
<td>0.001(0.008)</td>
<td>0.001(0.008)</td>
<td>0.001(0.008)</td>
<td>0.001(0.008)</td>
<td>0.001(0.008)</td>
</tr>
<tr>
<td>MG</td>
<td>1.134</td>
<td>0.496(0.496)</td>
<td>0.001(0.007)</td>
<td>0.001(0.007)</td>
<td>0.001(0.007)</td>
<td>0.001(0.007)</td>
<td>0.001(0.007)</td>
<td>0.001(0.007)</td>
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</tr>
<tr>
<td>TI</td>
<td>6.022</td>
<td>0.474</td>
<td>0.001(0.006)</td>
<td>0.001(0.006)</td>
<td>0.001(0.006)</td>
<td>0.001(0.006)</td>
<td>0.001(0.006)</td>
<td>0.001(0.006)</td>
<td>0.001(0.006)</td>
</tr>
<tr>
<td>MAD</td>
<td>22.653</td>
<td>16.233</td>
<td>50.932</td>
<td>0.144(0.288)</td>
<td>0.249(0.288)</td>
<td>0.066(0.198)</td>
<td>0.001(0.005)</td>
<td>0.002(0.008)</td>
<td>0.001(0.006)</td>
</tr>
<tr>
<td>GN</td>
<td>25.372</td>
<td>16.73</td>
<td>55.734</td>
<td>1.595</td>
<td>0.858(0.861)</td>
<td>0.503(0.826)</td>
<td>0.001(0.004)</td>
<td>0.003(0.004)</td>
<td>0.001(0.004)</td>
</tr>
<tr>
<td>DVM</td>
<td>33.43</td>
<td>55.336</td>
<td>69.12</td>
<td>1.548</td>
<td>0.564</td>
<td>1.000(1.000)</td>
<td>0.001(0.003)</td>
<td>0.003(0.006)</td>
<td>0.001(0.002)</td>
</tr>
<tr>
<td>RU</td>
<td>46.665</td>
<td>53.461</td>
<td>98.092</td>
<td>2.343</td>
<td>0.877</td>
<td>0.032</td>
<td>0.001(0.002)</td>
<td>0.001(0.002)</td>
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</tr>
<tr>
<td>OMAN</td>
<td>17.955</td>
<td>11.19</td>
<td>28.46</td>
<td>34.793</td>
<td>38.886</td>
<td>53.291</td>
<td>72.735</td>
<td>0.001(0.001)</td>
<td>0.001(0.001)</td>
</tr>
</tbody>
</table>

4.4.3 Genetic structure and origin of ghost net samples

A total of seven haplotypes (Lo1 n = 4, Lo4 n = 1, Lo42 n = 1, Lo44 n = 23, Lo97 n = 6, Lo98 n = 1 and Lo100 n = 1) were detected across the 770 bp fragment (n = 38) found in turtles entangled in ghost nets in the Maldives. Haplotypes Lo44 and Lo97 accounted for 79% of all ghost nets and represented haplotypes that are common in the east Indian population (Figure 4.5). 11% of all individuals caught in ghost nets belonged to haplotype Lo1, which is found in populations from east India, Sri Lanka, Australia and Oman. Two ‘orphan haplotypes’ (Lo98 and Lo100), which have not yet been reported from any population in the Indian Ocean, were found in our ghost net samples. In our bycatch samples from Madagascar, a total of four haplotypes (Lo1, Lo44, Lo97 and Lo67) were detected in the nine samples assessed. One of these haplotypes (Lo67 – Accession # MN342239) differed by one nucleotide mutation from the common Lo1 haplotype (Fig. 4) and accounted for >50% (n = 5) of the individuals tested.
We then utilised a mixed-stock analysis to determine the origin of olive ridley turtles caught in ghost nets throughout the Maldives (Figure 4.6). After excluding one orphan haplotype (Lo100; found in our ghost net samples but not the source populations), we ran a mixed stock analysis based on ghost nets found during the NE and SW monsoon seasons. The analysis resulted in very small and non-significant seasonal variations (Appendix 4.1) and we therefore compiled all our data to increase the overall robustness of our analysis (Figure 4.6a). Our results revealed a substantial contribution from east Indian (73%) and Sri Lankan stocks (23%). Contrary to our expectations, there was no evidence that entangled turtles originated from Oman as the

**Figure 4.5** Haplotype frequency chart comparing mixed stock (Total) and seasonal variation (South West (SW) and North East (NE) Monsoon) of the nesting olive ridley populations identified by our PCA and DA; Sri Lanka, Oman, Northern Australia (NTAus), Cape York Australia (CYPAus) and the east coast of India.
common Omani haplotype Lo99 was not present in the ghost net samples (Figure 4.5). When we included population estimates (Appendix 4.2) as a prior in our mixed stock analyses (Figure 4.6a), the contribution of the relatively small Sri Lankan population substantially reduced ($n = .002\%$) and east Indian population increased from 73% to 99% (Figure 4.6b). This result suggests that the majority of entangled turtles found in the Maldives archipelago originate from east Indian populations (Figure 6b).

**Figure 4.6** Mean relative contribution of 5 genetically distinct olive ridleys (*L. olivacea*) populations which were found entangled in ALDFG in the Maldives - estimated using a mixed-stock analysis (Bolker et al., 2007). Curves represent density probability functions. Panel (A) and (B) display results when estimations are based only on haplotype composition (A) or both, haplotype composition and population size (included as priors) (B). Abbreviations represent Australia Cape York (Australia CYP) and Australia Northern Territory (Australia NT).

### 4.4.4 Olive ridley mortality estimates in ghost nets

The average number of olive ridleys nesting annually in eastern India has been estimated at ~200,000 (Manoharakrishnan and Swaminathan, 2018), with a further 770 nesting in Sri Lanka (Rajakaruna et al., 2018). Indian and Sri Lankan nests hold on average 120 and 105 eggs, respectively (Manoharakrishnan and Swaminathan, 2018; Rajakaruna et al., 2018). If we
assume a hatching success rate of ~80% (Manoharakrishnan and Swaminathan, 2018), an estimated ~19,200,000 ‘new’ turtles are born every year in east India and ~64,600 in Sri Lanka. It has been established as a rule of thumb that 1 in every 1000 hatchling survives to a reproductive age (Frazer, 1983), so that would imply an annual net recruitment of around 19,200 turtles for east Indian and 64 for Sri Lankan populations. In this study, we found in only one year (2017 - 2018) 132 olive ridley turtles entangled in ghost nets in the Maldives alone. Based on our mixed stock analyses, we assumed that about 70% of these turtles originate from east Indian and 20% from Sri Lankan populations. Entanglement in ghost nets would therefore reduce yearly recruitment by 0.48% for the eastern Indian populations and by 41% for the Sri Lankan population.

3.5 Discussion

Drifting ghost nets represent a major threat to marine life but quantifying its impact on mortality rates of endangered species such as turtles remains a challenge. Here, we used a citizen science monitoring program to record ghost net turtle entanglements throughout the Maldives archipelago. In just one year (2017 - 2018), a total of 177 nets were recorded, which had 132 olive ridley turtles entangled within them. We analysed the mtDNA of 38 of these turtles and illustrated that entangled individuals originated predominantly from eastern Indian and Sri Lankan populations.

4.5.1 Impact of ghost nets on olive ridley populations

In contrast to a previous study, which found that 45% of all entangled turtles assessed could not be assigned to a known nesting population i.e. characterised as being ‘orphan haplotypes’
(Jensen et al., 2013), we only identified two orphans (equating to 5.3% of our entangled turtles). This therefore allowed us to trace (with high reliability) the origin of the majority of the entangled turtles found in the Maldives during the survey period. The result of the respective mixed stock analysis reveals that a large proportion (73% of the individuals assessed) originated from east India and 23% of the turtles from Sri Lanka. Australian and Omani source populations are relatively less impacted. Interestingly, if ‘source population size’ was included into our model calculations, the relative importance of the east Indian population increased to 99% (Figure 4.6). This highlights the importance of standardising such models when exploring estimations and assigning risk of given threats to certain populations. Currently, there is no consensus on how strongly population size should be weighted, and factors such as geographic distance may affect this decision. We therefore present the two extreme approaches (i.e. disregarding population size or giving it a strong weighting) and the true relative contributions of each source population is likely to lie somewhere between the two.

Surprisingly, we found no seasonal variation in the contribution of source populations but a decrease in the percentage of entangled turtles recorded during the SW monsoon (SW - 23%, NE - 55%). Ocean currents across the Maldivian archipelago change seasonally and are strongly associated with the monsoons. Therefore, we expected that different populations of turtles may be affected during different times of the year. One possible explanation for the lack of seasonal impact on relative haplotype frequencies is that olive ridley turtles (from east Indian and Sri Lankan rookeries) are using the Maldives as a foraging ground. This hypothesis is supported by the occasional observation of free-swimming olive ridleys in the Maldives (Anderson et al., 2009). Further support comes from the relative minor injuries found on a number of the turtles entangled, a result indicative of short time spans between entanglements and ghost net retrievals. Further still, neighbouring areas (Gulf of Mannar, Sri Lanka and the Arabian Sea) are known to be important foraging grounds for olive ridleys, findings based on
bycatch data and satellite telemetry of adult turtles (Kannan, 2008, Behera et al., 2018, Rees et al., 2012). Interestingly where juveniles and sub adults forage remain largely unknown (Bolten, 1993). If the Maldives is indeed an important foraging ground, and possibly one for juveniles and sub adults (based on the recorded size class distributions of the entangled turtles - see Appendix 4.3) urgent action should be implemented to try and manage this important habitat. However future research is needed to confirm that such grounds indeed exist.

Although it is difficult to exactly quantify the impact of ghost nets on regional populations, trying to estimate the impact can assist with management directives.

As discussed above, the population of olive ridleys nesting in eastern India and Sri Lanka were most at risk of entanglement in the nets found adrift in the Maldives. We estimate that yearly recruitment of olive ridleys may be impacted by ghost nets up to 0.48% for east Indian populations and as much as 41% for Sri Lankan populations. However, these estimates are only based on the recorded ghost nets, the majority from only two out of the 26 atolls and therefore neglect the fact that a large proportion of net entanglements were certainly missed by our team. Indeed, a recent study estimated that between 3400 and 12,200 entangled turtles may have gone undetected in the Maldives over a 51-month period (Stelfox et al., 2019). Moreover, better estimates on the levels of nest survivorship along the east coast of India and Sri Lanka may improve our impact assessment of ghost nets. Additionally, we do not consider ghost net entanglements close to Sri Lanka or the east Indian coast for which no data exists. Therefore, although our calculations above should only be read as rough estimates of the impact ghost nets have on olive ridley populations, they are likely underestimates of the true scale of the issue. Regardless of the accuracy, the threat is clear, an urgent need for more reliable population-ecological data is evident, which would allow for the improvement of ghost net impact assessments and support regional turtle conservation across the Indo-Pacific.
4.5.2 The importance of improving our phylogeography knowledge of olive ridleys (L. olivacea)

Our assessment of olive ridley phylogeography conformed to earlier analyses (Bowen et al., 1997, Shanker et al., 2004, Jensen et al., 2013), highlighting the existence of two distinct haplotype clusters (around Lo44 and Lo1, which are central to east India and Australia genetic stocks, respectively). The current line of thought gives two hypotheses explaining which of these haplotype clusters is the ancestral lineage. The first (Bowen et al., 1997, Jensen et al., 2013) suggests that all Indo-Pacific rookeries evolved from haplotype Lo1 (found in all populations in this study, including ghost net and bycatch samples) and that radiation of Lo44 subsequently happened in India. The alternative hypothesis states that the east Indian haplotype Lo44 is the ancestral lineage and that Lo1 represents a successful descendent (Shanker et al., 2004). The latter hypothesis is supported by the presence of a signature 7 bp INDEL in haplotype Lo44 that is also found in Kemp ridleys (Lepidochelys kempii) and many other species of sea turtles (loggerhead, green, hawksbill and leatherback). Our analysis points to Lo1 being the ancestral haplotype giving further support to hypothesis one above, as the cluster surrounding this haplotype show greater deviation from a star-shaped pattern, an indication in itself of a longer evolutionary history (Slatkin and Hudson, 1991). However, it is beyond the scope of this study to determine which of the hypotheses are true.

Interestingly, our study did however identify that haplotype composition found in the Omani population were unique to this genetic stock. Both Lo1 and a derivative of Lo44 were present in equal frequencies. It is highly unlikely that both of these haplotypes evolved in parallel in Oman, because both the phylogeographic hypotheses highlighted above require some sort of dispersal event. Consequently, the current genetic structure of the Omani population strongly suggests that there were two distinct colonisation events. This is also supported by an analysis of genetic and geographic distances of different populations (Appendix 4.7). While there is a
clear positive relationship for Australian and eastern Indian populations, indicating that distance affects the degree of genetic similarity, no such relationship was found for the Oman population. The same is true for Sri Lanka, suggesting that both locations were colonised by at least two different haplotypes, perhaps from distant populations.

Such long-distance migrations have recently been hypothesised, based on the detection of eastern Pacific haplotypes in Australian and east Indian populations (Shanker et al., 2004, Jensen et al., 2013). In our study, we also found one orphan haplotype from a female individual (Lo100), which differs by only one mutation from rookeries in Birdshead Peninsula, Papua, Indonesia (Jensen et al., 2013). This indicates one of two possibilities. Either, this individual drifted entangled in a ghost net from Indonesia, or it represents a migrant visitor implying a low-volume influx of haplotypes from distant populations. Recent sighting of an olive ridley turtle off the south coast of the UK is further evidence for migrancy or individuals simply losing their way (https://www.bbc.co.uk/news/uk-england-sussex-51167213).

Finally, we want to highlight the urgency to investigate uncharacterised rookeries, e.g. along the west coast of India (Shanker et al., 2004), Kenya (Frazier, 1975, Okemwa et al., 2004), Yemen (Frazier, 1980) and Andaman and Nicobar Islands (Andrews et al., 2006). Such data would be crucial to define population structure in the region. For example, our ‘random’ analysis of bycatch data from French longliners (collected off the coast of Madagascar), revealed a haplotype previously described by Bowen et al., (1997) from rookeries in the Atlantic (Lo67). In order to interpret such findings and to determine the importance of long-distance migrations for conservation, it would be crucial to fill these gaps in the map of olive ridley phylogeography.
4.6 Conclusions

Here we highlight that ghost nets may threaten the existence of small turtle populations and its quantification and management should be a major focus of the Northern Indian Ocean Marine Turtle Task Force of the Indian Ocean, South East Asia Marine turtle Memorandum of Understanding (NIO-MTTF IOSEA-MoU). Specifically, we identify Sri Lankan rookeries, which are genetically distinct from other neighbouring rookeries and have relatively small nesting numbers to be at high risk from ghost nets in the Indian Ocean.

We also show that citizen scientist is an invaluable asset to research and highlights the crucial contribution that citizen science projects can play in conservation and academic research. The Olive Ridley Project have a network of informed citizens scientist that are able to report ghost gear and entanglement events over a broad geographical region and respond quickly to entanglement reports using minimal resource. Citizen science can be a useful tool to help inform conservation practitioners and should be included in methodological designs where appropriate.

Managing the impacts of ghost gear in the Indian Ocean will require a multifaceted approach. First, all ghost nets reported in this study were unidentified, damaged fragments and therefore had no clear link to a specific fishery. Therefore, regional fishery managers, such as the Indian Ocean Tuna Commission (IOTC), need to improve compliance where appropriate and/or implement gear marking and gear loss reporting by its country members. Second, the development of incentivised deposit return schemes (in ports or landing sites) would undoubtedly encourage/improve appropriate disposal of end of life and/or damaged fishing gear. Finally, educational workshops must run in parallel to any management strategy in order to better outline reasons for gear loss and prevent loss in the future. Informed fishers are more
likely to abide by new or existing policies when information is available about the impact of ghost gear to their livelihoods.

We end by highlighting that ghost gear drifting into the Maldives impacts a number of source populations (i.e. genetic stocks) of olive ridleys from different countries. Further the gear lost may originate from even more countries in the region and beyond. Therefore, it is imperative that future conservation and research efforts which aim to tackle this issue involves a close collaboration between NGOs, regional fisheries managers and governments from all countries within the Northern Indian Ocean and ideally even further.
References


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ftp://192.218.129.11/pub/CRAN/web/packages/MASS/MASS.pdf


Appendix

Appendix 4.1 Estimated source contribution from five nesting populations to ALDFG found during the NE and SW monsoon in the Maldives. Figure S4a was run without estimated population priors and S4b was run with estimated population. Population estimates were reported according to supplementary Information S1. Bars represent 95% confidence
<table>
<thead>
<tr>
<th>Rookery name</th>
<th>Abbreviation</th>
<th>n</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>East coast of India</td>
<td>India</td>
<td>170,939</td>
<td>Chandrana, R., M. Manohararishnan and K Shanker. Long-term Monitoring and Community-based Conservation of Olive Ridley Turtles in Odisha. CMPA Technical Series No. 7. Indo-German Biodiversity Programme, GIZ-India, New Delhi</td>
</tr>
</tbody>
</table>
Appendix 4.2 Estimated population size (n) according to historical data. In this study we used Limpus. 2008, population estimates based on the lower figures according to the distribution of breeding sites in the Indian Ocean figure presented in the review. * Based on average of 2 nests per individual and a yearly average of 770 nests

Appendix 4.3 Frequency distribution of size class of *Lepidochelys olivacea* found entangled in ALDFG in the Maldivian archipelago (n= 37).
Appendix 4.4 Variable sites (770 bp fragment length) for all published mtDNA haplotypes for *Lepidochelys olivacea* rookeries in India, Australia, Oman and Sri Lanka. New haplotype names are labelled as “New Hap” and old names are shown as “Old Hap”. See Figure 2 and 3 to identify haplotype to nesting, bycatch or ghost net samples.

| Lo1**       | J | G | T | C | T | G | G | G | C | T | G | T |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| Lo2**       | G | G | T | C | T | G | G | G | C | T | G | G | T |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| Lo3**       | H | G | T | C | T | G | G | G | C | T | G | G | T |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| Lo5**       | G | T | C | C | T | G | G | C | T | G | G | T |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| Lo15**      | J | G | T | C | T | G | G | C | T | G | G | T |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| Lo21**      | G | T | C | C | T | G | G | C | T | G | G | T |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| Lo27**      | M | T | C | T | T | G | G | T | G | C | T | G | T |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| Lo42**      | I | G | T | C | T | G | G | C | T | G | G | T |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| Lo44**      | K | G |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| Lo46**      | N | T | C | T | T | G | G | G | C | T | G | T |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| Lo47**      | K1| A | G | G |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| Lo48**      | K2| G |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| Lo49**      | K3| G | C |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| Lo50**      | K4|   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| Lo51**      | K5| G |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| Lo67**      | F | G | T | C | T | G | G | G | C | T | G | T |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| Lo97**      | G |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| Lo98**      | G |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| Lo99**      | G |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| Lo100**     | T | C | A | T | G | G | G | C | T | A | C | C | G | G | T |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |

*a Shanker et al. 2004, b Bowen et al. 1997, c Jensen et al. 2013 and d This study.*
Appendix 4.5 Variable sites for haplotypes that are identical at the short 410bp fragment (red) but show divergence at the longer 770 bp fragment (black). New haplotype names are marked as “Long Hap”.

<table>
<thead>
<tr>
<th>Consensus ID</th>
<th>Lo15 J (Lo1)</th>
<th>Lo97 K (Lo44)</th>
<th>Lo98 K4 (Lo50)</th>
</tr>
</thead>
</table>
Appendix 4.6 Genetic pairwise comparisons of nucleotide difference in mtDNA of *L. olivacea* after grouping of rookeries along the east coast of India and those in Northern Australia.

<table>
<thead>
<tr>
<th></th>
<th>CYP Aus</th>
<th>NT Aus</th>
<th>INDIA</th>
<th>OMAN</th>
<th>Sri Lanka</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP Aus</td>
<td>0.002 (0.005)</td>
<td>0.001 (0.005)</td>
<td>0.001 (0.005)</td>
<td>0.001 (0.005)</td>
<td></td>
</tr>
<tr>
<td>NT Aus</td>
<td>11.97</td>
<td>0.001 (0.004)</td>
<td>0.001 (0.004)</td>
<td>0.001 (0.004)</td>
<td></td>
</tr>
<tr>
<td>INDIA</td>
<td>76.56</td>
<td>261.99</td>
<td>0.001 (0.003)</td>
<td>0.001 (0.003)</td>
<td></td>
</tr>
<tr>
<td>OMAN</td>
<td>20.75</td>
<td>55.7</td>
<td>116.22</td>
<td>0.001 (0.002)</td>
<td></td>
</tr>
<tr>
<td>SL</td>
<td>6.4</td>
<td>25.91</td>
<td>18.16</td>
<td>20.14</td>
<td></td>
</tr>
</tbody>
</table>

Pairwise F values (below diagonal) and Pairwise p-Value (above diagonal) among 5 rookeries after consolidation of eastern Indian rookeries. Based on the 410 bp sequence of the control region of the mtDNA. Abbreviation for each site is given in supplementary Information S1.
Appendix 4.7. Relationship between geographic distance and genetic distance amongst 4 broad nesting populations for olive ridley turtles (*L. olivacea*). Oman and Sri Lanka nesting sites are compared with all other nesting sites. Coloured bands represent confident intervals of regression analyses.
Chapter 5: General discussion and key findings

5.1 Introduction

The aim of this thesis was to improve our knowledge around the impact of abandoned, lost or discarded fishing nets (or ghost nets), which are found drifting into the Maldives and often have entangled turtles within. This improved knowledge would then provide the Olive Ridley Project (ORP), a registered charity for which I am the CEO, the correct tools to make informed decisions with the larger goal of tackling this pollutant head on. I begin this chapter by first summarising the key findings of my research and discuss how this thesis builds knowledge of the issue. I then discuss how my research has been applied by the broader scientific community and look at how the research could extend and be replicated outside of the Maldives. I also provide future recommendations and highlight how my research will provide the foundations for management decisions within the Olive Ridley Project.

5.1.1 Key findings

One of the major challenges when assessing ghost gear and associated ghost fishing is its cryptic and transboundary nature (Gilman, 2015). These challenges make it difficult to identify a source of the pollutant, conduct quantitative analyses and/or estimate mortality rates. Without tools to unlock this information it becomes difficult to assess the effectiveness of existing and new management plans put in place to combat the issue. This thesis identified key gaps in the literature and develops detailed methods that can be replicated or built upon by the scientific community. It is hoped that the detailed methods outlined in this thesis provides the scientific community and fishery managers with a set of tools that can be used as a starting point to assess
the impact of ghost gear to target species and/or assess gear loss from different fisheries around the world. This thesis was able to fill in a number of research priorities and knowledge gaps with in the field.

In Chapter 1 I was able to identify regional data gaps, namely the Southern, Arctic and Indian Oceans and highlighted that ghost gear entanglements were reported in all animal groups analysed. One major discovery gleaned from this review was the necessity to distinguish between active versus non-active fishing gear entanglements. Moreover, I was able to uncover the adhoc and scattered reporting style of entanglement events between many institutes, biologists, diving groups, NGOs and local governments. In order to make any assumptions on the impact ghost gear may have at the population level we must first consolidate these data reporters for each ocean basin and target animal group so that we can greatly improve our understanding of ghost gear interactions.

5.1.2 Uncovering the cryptic and transboundary nature of drifting ghost gear in the Maldives

By using a combination of statistical (Chapter 2) and oceanographic (Chapter 3) modelling I was first able to discover that mesh size, monsoon season, presence/absence of floats and net colour are key drivers that increases the likelihood of sea turtle entanglement. I was then able to identify a putative origin of ghost gear found in the Maldives. Whilst the majority of ghost gear encounters and entanglement events are widely reported as transboundary (Laist and Wray, 1995, Gunn et al., 2010, Stelfox et al., 2015, Wilcox et al., 2015, He & Suuronen, 2018). Clustering models developed in Chapter 2 were able to broadly assign ghost gear types to fisheries. Further evidence and improved resolution of responsible fisheries was made possible through analysing drift trajectories in Chapter 3. I concluded, for the first time that purse seine fisheries operating in the western Indian Ocean (predominantly Spanish and French) and small scale, coastal gill net fisheries in the Northern Indian Ocean were contributing to ghost gear
found in the Maldives. Additionally, short drift times suggest a proportion of ghost nets found in the Maldives are coming from illegal, unreported and unregulated fishing within the exclusive economic zone of the Maldivian archipelago. Bioaccumulation percentage cover and size of the goose neck barnacle *Lepas anatifera* provides a novel method to estimate minimum drift times of ghost gear and provides a platform for other researchers in different regions to build upon. An indirect and unanticipated finding from ageing ghost gear was that the potential that ghost gear has to introduce new and invasive species to delicate reef systems.

5.1.3 The impact of ghost gear in the Maldives

Attempting to analyse the impact of ghost gear to sea turtle populations have proven difficult in the past (e.g. Jensen et al., 2013). Often a lack of knowledge on the phylogenetic structure of nesting females makes it difficult to accurately assign individuals from a mixed genetic stock to a source population. However, in Chapter 4 I was able to build on olive ridley sea turtle phylogenetics in the Indian Ocean by expanding genetic sampling into Oman and increasing the resolution of nesting females along the east coast of India. I was then able to infer that the majority of entangled turtles found in the Maldives belong to the east Indian and Sri Lankan population. Estimates in mortality rates revealed that whilst ghost gear may only have a minimal impact to the east Indian population (impacting 0.48% of yearly recruitment) the neighbouring and unique genetic stock of Sri Lanka may be more severely impacted (impacting 41% of yearly recruitment). These estimates provide for the first time a quantitate analyses of the impact of ghost gear to sea turtles found entangled in the Maldives. It is hoped that methods used in Chapters 2, 3 and 4 are able to be replicated and built upon so that ghost gear can be accounted for in future management strategies around the world.
5.2 Three years later: a global impact.

Since my literature review was published (Chapter 1, Stelfox et al., 2016) it has grasped the attention of the scientific community from all around the world and advanced our knowledge of abandoned, lost or discarded fishing gear (ALDFG) in general. An unanticipated outcome of this review was the beginning of a broad scale discussion between researchers from different disciplines on the need for accurate reporting and improved data collection. This resulted in a series of published articles associated with a wide range of different animal groups and across a wide geographical range.

5.2.1 Reporting ALDFG events and the importance of entanglement discrimination.

The cryptic and transboundary nature of ghost gear (reported in Chapter 3) makes monitoring the occurrence and impact of ghost fishing challenging. As a result, research focusses on the economic cost of ghost gear (Al-Masroori et al., 2004, Gilardi et al., 2010) rather than the impact at the population level (see Chapter 4 and Wilcox et al., 2015). Therefore, ghost gear may directly impact the survival of species and/or certain populations. Especially if they (the species or populations) are under other anthropogenic pressure which may undermine existing conservation management plans. Undoubtedly, ghost gear has been reported to impact a wide diversity of fauna (see Chapter 1 and Laist, 1995). However, a major caveat to the majority of ghost gear research, particularly if the entanglement was not observed directly, is not knowing if entanglement is due to interaction with active fishing gear (i.e. bycatch) or abandoned lost or discarded fishing gear (i.e. ghost gear). Certainly, bycatch is widely regarded as a major threat to marine megafauna (Read et al., 2006, Žydelis et al., 2009, Lewison et al., 2014, Temple et al., 2019), however ghost gear impact assessments are regularly overlooked and therefore claims that ghost gear is not a primary issue (Asmutis-Silvia et al., 2017) should be avoided until more research is available. Specifically, Asmutis-Silvia et al. (2017) published a
rebuttal that claimed that the review in this thesis (Chapter 1) had misinterpreted cetacean entanglements in ALDFG and that this misinterpretation may detract from conservation efforts associated with the issue of entanglement in active gear, i.e. bycatch. However, my review (Chapter 1) clearly explained how ALDFG entanglements were characterised and this contradicts Asmutis-Silvia rebuttal (Stelfox, 2017). Interestingly, Asmutis-Silvia et al. (2017) rebuttal strengthened my recommendation that researchers need to carefully discriminate between bycatch and other causes of entanglement and provide a detailed report of each entangling material where possible (as identified in Chapter 1). In light of my recommendations a series of published articles shortly followed, all of which either support my conclusion or fill knowledge gaps highlighted in Chapter 1 (de Quirós et al., 2018; Halsband et al., 2018, He and Suuronen, 2018, Richardson et al., 2019, Parton et al., 2019).

5.2.2 Shark entanglements in ALDFG: whats next?

My review (Chapter 1) also highlighted substantial data gaps surrounding ALDFG and with elasmobranch entanglements in particular and I indicated the need for more research in these areas. Ward-Paige and Worm, (2017) global assessment, which utilised diver surveys also found significant data gaps but highlighted the threat of ghost gear was ranked as high risk in most cases. A later study following on from my review (Chapter 1) focussed on a global review of shark and ray entanglement using a novel approach of published literature and social media reviews (Parton et al., 2019). In this study they found that ghost gear is the most common entangling objects and as a result have developed the ‘Shark and Ray Entanglement Network’ (Sh.a.R.E.N.) in collaboration with the Shark Trust (https://www.sharktrust.org/shark-and-ray-entanglement-network) and the University of Exeter. Moreover, my review (Chapter 1) provided enough evidence for the International Seafood Sustainability Foundation (ISSF) to design and implement fish aggregating devices (FADs) aimed at reducing shark entanglement by ghost fishing. This was highlighted in the 3rd meeting of the FAD management option
intersessional working group hosted by the Western and Central Pacific Fisheries commission, in the Republic of the Marshall Islands in 2018 (Moreno et al., 2018).

5.2.3 ALDFG global hotspot bias: are we moving in the right direction?

The urgent need for a centralised data collection protocol, following a minimum global criterion, was a major point highlighted in my review (Chapter 1). Improved data recording would help highlight hotspot areas and their impacts on different animal groups on a global scale. Shortly after publishing this review (Stelfox et al., 2016, Chapter 1) I presented my analyses at the Global Ghost Gear Initiative (GGGI) annual general meeting, a global sectoral alliance of fishing industry, private sector, corporates, NGOs, academia and governments, which are all focussed on solving the problem of ALDFG worldwide. My suggestion to centralise data collection contributed to the development of a central data portal for ALDFG worldwide and is now the largest of its kind in the world (https://globalghostgearportal.net/dp/usermanagement/login.php).

Geographical area was also highlighted as a major bias in my review (Chapter 1), as the vast majority of research was in the Pacific and Atlantic Ocean. A more recent analysis of pinniped entanglement in plastics also corroborate with this finding (Jepsen and Nico de Bruyn, 2019). However, Norway have acknowledged this geographical bias and have recently created the “Plastics in the Arctic program” which began in 2018 and will continue until 2023 (Halsband et al., 2018). The purpose of this programme is to collect data on all plastics from macro (including ghost gear) to nano plastics in the Arctic and build evidence on the issue in the region (Halsband et al., 2018). This study will be an important contribution to the understanding of ghost gear in arctic systems and marine plastics and will improve our knowledge on the impact of ALDFG on Arctic systems.
5.3 Olive Ridley Project: building model accuracy through citizen science

Citizen science can be defined as the collection of data by the general public for a specific research project (Irwin, 2002). This method allows researchers to extend their reach into a wider geographical area and increase data volume using minimal resources and at very low costs. Citizen science projects are now widely used to investigate various ecological systems (Evans et al., 2005, Williams et al., 2015, Sullivan et al., 2015, Nelms et al., 2017) and in recent years an increase in the number of citizen science published reports have been reported (Silvertown, 2009). The data collected by ORP’s citizen scientists formed the foundations for this thesis, which allowed me to investigate ghost gear and turtle entanglements in more detail (Chapter 2, 3 and 4). Increasing the volume of data can improve model accuracy and estimates.

5.3.1 Incentivised data submission

Data reporting on ghost gear is not as glamorous as megafauna citizen science projects such as reef mantas (*Mobula alfredi*, Armstrong et al., 2019), therefore additional incentives must exist to maintain interest and encourage long-term contributions. To overcome these challenges, I plan to incentivise citizen scientists by providing immediate feedback on the data they are submitting via the ORP website (see for example www.seagrassspotter.org). This would reinforce their reason to submit data and hopefully encourage consistency over time. To do this I plan to embed statistical models built in Chapter 2 along with the barnacle capitulum length (Chapter 3), onto the ORP website providing real-time analyses of the data submitted. After submission via the online portal the user will be provided with a printout, giving information that includes risk analyses of the net and broad classifications of potential fisheries it belongs too. Moreover, if capitulum data are available, the printout will also provide an
estimated drift time and a map showing potential drift trajectories and putative origin. This printout will be downloadable and can be shared amongst peers to encourage additional participants into the project. The feedback will hopefully encourage consistent data collection and help improve model accuracy by lowering false positive rates found in our current models (Chapter 2).

5.3.2 Extending data collection into the Indian Ocean.

It is also hoped that data collection can now extend beyond the Maldives so that models (as in Chapter 2) can be scaled up to incorporate ghost nets throughout the Indian Ocean. To achieve this ORP will be collaborating with the Convention on the Conservation of Migratory Species of Wild Animals. ORP has joined the Memorandum of Understanding on the Conservation and Management of Marine Turtles and their Habitats of the Indian Ocean and South-East Asia (IOSEA Marine Turtle MOU) and its associated Conservation and Management Plan (CMP) whose main goals are to encourage Indian Ocean cooperating governments and NGOs to contribute data on ALDFG. This proposal will first be submitted as a joint ORP/IOSEA Secretariat paper under item 10.5 (Other Opportunities for Collaboration) at the 8th meeting of signatories to the MoU on the Conservation and Management of Marine Turtles and their Habitats of the IOSEA scheduled in October 2019 (Appendix 5.1). Through this collaboration, I hope to provide a platform for improved ghost net and turtle entanglement reporting in the Indian Ocean, where data on these events are significantly lacking worldwide (see Chapter 1). Further such a resource will provide open access data to regional fisheries managers such as the Indian Ocean Tuna Commission (IOTC) to better manage the issue.
5.4 Not so cryptic: a call to action to address the transboundary nature of ghost nets in the Maldives

This thesis provides the foundations to better understanding the ghost net issue in the Maldives and, for the first time, uncovers a putative origin from the wider Indian Ocean (see Chapter 2 and 3). Clearly, a proportion of ghost nets entering the Maldives are coming from a wide variety of fisheries (identified in Chapter 2) and they likely drift through multiple maritime borders before being found in the Maldives (identified in Chapter 3). However, identifying specific fisheries with absolute accuracy using these methods alone is unlikely and we must explore additional tools to help combat the issue in the Maldives.

5.4.1 Gear marking as a method to identify net types.

Gear marking was initially discussed at an expert consultation by the Food and Agriculture Organisation (FAO) in 1991 in Canada (FAO, 1991). It was again discussed in Rome in 2016 (FAO, 2016). Since this time the Committee on Fisheries (COFI) have endorsed the work and requested further development of the draft guidelines of gear marking, encouraging the FAO to facilitate the implementation of gear marking for Indonesian gill nets and globally for FADs (Toole, 2017). However, in the Maldives ghost nets are often found as individual net fragments (see Chapter 2) or an amalgamation of these fragments to form huge conglomerates (Figure 5.1). Therefore, future gear marking efforts must allow for the identification of partial net fragments from a diverse range of fisheries in the region. Moreover, our ocean simulations (Chapter 3) indicated that an unknown proportion of nets found in the Maldives are originating from coastal zones, i.e. artisanal fisheries, therefore gear marking projects should be cheap or free to the fishers. Additionally, utilising new technology to combat gear loss (e.g. Blockchain
technology as suggested in **Chapter 2** should be explored to provide consumer driven changes within the gill and trawl net fisheries, as these are the predominant net fragments found in the Maldives (see **Chapter 2**).

![Figure 5.1](image1.jpg)

**Figure 5.1** Ghost nets found in the Maldives vary in size from small fragments (left) to large conglomerates of different fragments of net types (right). Three olive ridley sea turtles were found entangled in the small fragment (black arrows) Photo credit: Laura Whiteley

5.4.2 *Fish Aggregating Devices (FADs) management in the Maldives.*

Purse seine fisheries operating from the Seychelles, Spain, France, Korea and Mauritius and their associated drifting fish aggregating devices (dFADs) have been identified in this study as high-risk fisheries likely contributing to the ghost gear issue in the Maldives (see **Chapter 3**). Typically, dFADs in the Maldives are found as complete systems (Figure 5.2), with trackers attached to a bamboo platform. The fishing net portion of the ghost dFAD (now referred to as ghost net) are made from scrap purse seine nets and hang on the underside of the bamboo raft.
Figure 5.2 Fish Aggregating Device (FAD) found in Laamu atoll in the Maldives with attached tracker (left). Entangled olive ridley found in a FAD (arrow) in a separate case in the Maldives found in North Male (right) Photo credit: Jenni Choma

Evidence of dFADs drifting into the Maldives are supported by our ocean current simulations in Chapter 3, statistical clustering of FADs from nets found in the Maldives and from reported observations from citizen scientists (Stelfox et al., 2015). Major Purse seine vessel origin operating in the Indian Ocean are France and Spain (Davies et al., 2014), moreover ocean current simulations suggest a putative origin of ghost nets in areas that overlap France and Spain purse seiners across both monsoons (Chapter 3). Therefore, to address these issues a project similar to ‘FAD watch’ (Zudaire et al., 2018) that has been set up in the Seychelles, would also benefit the Maldivian archipelago (as suggested in Chapter 3). Pole and line fishers in the Maldives use dFADs as fishing grounds (Per. Obvs) therefore the FAD watch programme must incorporate fishers from the Maldives to help extend observer coverage and allow for recovery of some of the gear. This project is in the early stages of dialogue between OPAGAC (Spanish purse seiners), the Government of the Maldives and ORP. The results from Chapters 2 and 3 will form the foundation for selecting area to pilot the project and reason to initiative just such a project in the first place.

5.4.3 Education may be the key to change.
Identifying the reason why fishing gear is lost in the first instance is beyond the scope of this thesis, however a recent survey in Northern Australia suggests that snagging of nets and gear conflicts were the two main reasons for gear loss as a result of overcrowding, overcapacity and illegal, unreported and unregulated (IUU) fishing (Richardson et al., 2018). The economic cost of losing fishing gear can be great (Scheld et al., 2016, Bilkovic et al., 2016), therefore fishers are under tremendous pressure not to lose their gear, particularly small-scale artisanal fishers with limited resources. Educating coastal fishers on the benefit of proper gear disposal, gear management and providing an alternative income outside of fishing may help highlight the importance of reducing fishing pressure and safeguarding local stocks for future generations. Certainly, ORP have had great success in Pakistan working with artisanal fishers to dispose of end of life gear and retrieve ghost gear, turning them into products such as bracelets, dog leashes and donkey harnesses that they sell to generate an alternative income. Through this program that started in 2016, the community have collected and recycled 4 tons of ghost nets and generated income that has sustained families during low fishing season. The ORP also report reduce fishing activity from a number of fishers that now focus more time on collecting ghost gear and generating more income than fishing. Moreover, education to consumers will help drive out IUU fishing by encouraging sustainably sourced fish from reputable and local sources. Particularly amongst the younger generation who are the world future leaders and decision makers. To this end, I have co-authored a children’s story book called ‘The Adventures of Olive the Sea Turtle’. This story follows the many challenges faced by Olive as she journeys from the nest to the open ocean (see Appendix 5.2 for an example of illustrations). This book is written as an adventure story and aims to connect children to nature, it will be published by Silver-Goat media (https://www.silvergoatmedia.com/) and available toward the end of 2019 in English, Spanish and French.
5.5 The Olive Ridley (*Lepidochelys olivacea*): it’s not just about rehabilitation.

Collectively, the amount of ghost nets analysed over a 51-month study period (see Chapter 2) may be responsible for entangling 3,400 to 12,200 turtles. From this we can infer that only 1 to 4% of cases are detected by organisations such as ORP in the Maldives (according to the 131 turtles found during this study period). Therefore, the majority of entanglements go undetected. Moreover, genetic analyses conducted during this thesis highlighted that turtles found entangled in the Maldives originate from Indian and Sri Lankan genetic stocks (see Chapter 4), lending further support that ghost nets can be transboundary and require collaboration between multiple stakeholders. Interestingly, entanglement injuries vary from minor cuts and scrapes to deep lacerations and amputations (Figure 5.3). This suggests that sea turtle exposure to ghost net entanglement vary and whilst some turtles may have drifted entangled far from the Maldives others may be entangled close to or within the EEZ of the Maldivian archipelago.

5.5.1 Sri Lanka olive ridleys: further population analyses as a matter of urgency

The Olive Ridley Project provides rehabilitation for entangled turtles in the Maldives, however the number of turtles cared for at any given time (*n* = 8) is small and unlikely to have a major impact at a population level. Despite the importance of rehabilitation in the context of conservation and animal care, research priorities should be given to olive ridley populations in Sri Lanka about which very little is known, as there may be impacted most by ghost nets found in the Maldives (see Chapter 4). This thesis estimated that approximately 33% of yearly recruitment from Sri Lankan olive ridley populations may be impacted by ghost nets (evidenced in Chapter 4). It is important to quantitatively establish other threats to this small
but genetically distinct population, in order to provide more accurate population assessments and nest predation rates. This call to action will be presented at the Northern Indian Ocean Marine Turtle Task Force (NIO-MTTF) established by the CMS IOSEA Marine Turtle MOU. NIO-MTTF includes country members from the Maldives, Pakistan, Bangladesh, Sri Lanka and India, working together to protect sea turtles in the Indian Ocean. My work indicates that such cross sectorial collaboration needs to be encouraged and developed in order to better record and manage ghost nets, specifically to share resources that would allow Sri Lanka to conduct more research on its country turtle populations.

5.5.2 Foraging ecology of olive ridleys found entangled in the Maldives.

I have demonstrated in this thesis that olive ridley turtles become entangled in ghost nets from a broad geographic region (see Chapter 4), however it is unknown if they are entangled close to the Maldives (short drift times) or closer to the population from which they came (long drift times). Long distance travel has certainly been observed in Indian populations as a result of flipper tags from Indian populations being found in Sri Lanka (Pandav and Choudhury, 1998). However, most observations on sea turtle movement are based on adults (Tripathy and Pandav, 2008, Whiting et al., 2007) and juvenile and sub-adults’ movements are less well known. Entanglement injuries for olive ridleys in the Maldives suggest that a proportion of turtles may have become entangled within the EEZ of the Maldives (evidenced in Chapter 4). This highlights that juveniles and sub-adults from India and/or Sri Lanka may be using the EEZ of the Maldives as a foraging ground. Indeed, the majority of turtles found in the Maldives are juveniles or sub-adults (see Chapter 1) and this provides a unique opportunity to investigate this hypothesis further. Based on my hypotheses presented in Chapter 4, ORP will now begin a satellite-tagging programme to investigate the foraging ecology of juvenile and sub-adult olive ridleys found within the Maldives. Permits from the Environmental Protection Agency
(EPA) are currently underway (August 2019). I am also in early discussion with the technology department of the BBC to design satellite tags for rehabilitated turtles so they can be tracked and followed by millions of viewers for a new TV series on the Western Indian Ocean, due to be filmed next year (October 2020).

5.6 Conclusion

The overall aim of this thesis was to explore the origin of abandoned, lost or discarded fishing nets (or ghost nets) and their impact on turtles in the Maldivian archipelago. I wanted to use this information as a management tool for the Olive Ridley Project (ORP) to help inform how resources should be allocated to tackle the issue.

The literature review (Chapter 1) highlighted that much of what we know about ALDFG comes from work carried out in the Atlantic and Pacific. However, the Indian Ocean was an underrepresented study area, firmly cementing the issues faced around ghost gear in this region the need for this thesis, and strengthening the charitable objectives of ORP.

Chapters 2 and 3, provided the foundations for tools that will now (hopefully) provide immediate feedback to citizen scientists using the ORP online portal, and further improve accuracy through consistent data submission. Moreover, capitulum length of the goose necked barnacle, Lepas anatifera provides a novel method to estimate minimum drift times and this will provide additional information that can accompany ghost net data. However, at present barnacle size can only provide age estimates up to 105 days. Further analyses should therefore focus on capturing maximum barnacle size and growth rates in different environments (e.g. temperature) for barnacles and biofouling communities in pelagic environments. Moreover,
documenting different diatom communities within the Indian Ocean (Chapter 3) may also help uncover regional specialisation of communities and provide additional evidence as to which countries the ghost nets drift too.

The lagrangian simulations (Chapter 3) show that purse seine fisheries operate in areas of high gear loss. Moreover, the simulations highlight the transboundary nature of ghost nets and allows us to uncover the cryptic nature of nets in the area. Additionally, small-scale fisheries make up an important sector responsible for substantial gear loss. My simulations suggest that Indian and Sri Lankan coastlines are ‘high risk’ areas and this analysis highlights the need for ORP to focus in these areas. Working with fishing communities to set up gear disposal facilities and provide an alternative income outside of fishing may help encourage better gear management and gear recovery. Moreover, educating the community will allow for better adoption, should gear marking or similar projects be implemented in the future. Although outside the scope of this thesis further research into the overlap between fisheries and sea turtle habitats should be explored. For example, Euguchi et al. (2017) confirmed the effectiveness of temporary closures of gill net fisheries during peak leatherback turtle bycatch season in California. It was shown that temporary closures are the most effective way to protect sea turtles whilst continuing fishing. This type of overlap between sea turtle habitats and fisheries could provide additional evidence as to the likelihood of turtle entanglement in ghost gear from different fisheries.

Finally, olive ridley populations most impacted by ghost nets in the Maldives are those from east India and Sri Lanka (Chapter 4). Moreover, the impact on yearly recruitment on Sri Lankan populations is significant (33%) and further highlights the urgent need to improve analysis of turtle population and mortality rates in the country. There is a general lack of knowledge on turtle movements, especially for juvenile and sub-adult olive ridleys, and future research should focus on this to uncover potential turtle entangling hotspot and high-risk areas.
References


Toole, J., 2017. FAO Effort on Combating Abandoned, Lost or Otherwise Discarded Fishing


Appendix

Abandoned, lost or discarded fishing nets: a need for improved quantitative analyses in the Indian Ocean

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Abstract

Sea turtle entanglements in abandoned, lost or discarded fishing nets (ghost nets) have been recognised as a major mortality source for sea turtles. However, little effort has been made to assess the impact, ghost nets have at the population level. Such limited research is likely driven by; (a) the effort required to quantitatively assess the number of ghost net entanglements and (b) methodological difficulties in tracing the origin of entangled individuals. Here we provide one solution to try and overcome these issues, principally with the application of a large-scale citizen science approach aimed at recording ghost nets and turtle entanglement throughout the whole Indian Ocean. Specifically, we propose to build upon an existing database which will allow us to quantitatively assess the impact ghost nets have on sea turtles and their habitats.
Introduction

Abandoned lost or otherwise discarded fishing gear (ALDFG) or ‘ghost gear’ as it is more commonly known is estimated to account for around 10% of marine debris worldwide (Macfadyen et al., 2009) which was already estimated at around 640,000 tons. It is likely that this is an underestimation since studies exploring the amount of marine litter (and ghost gear in particular), use a variety of different methods to measure; including volume, weight and/or abundance, making comparisons between studies difficult. Further, estimates do not currently include gear coming from illegal, unreported and unregulated (IUU) fishing practices and those countries that do not record gear loss.

Despite this lack of somewhat basic knowledge on the amount of ghost gear floating in our oceans, entanglement in marine debris has been recognised as a major threat to many marine animals including, seals, whales, dolphins and turtles (Laist et al., 1987; Baulch et al., 2014, Stelfox et al., 2016). Sea turtles in particular have been recognised as one major or key species specifically vulnerable to marine debris and this is complicated by their complex behaviour, seasonal migration and foraging ecology (Heppell et al. 2002; Bolten, 2003; James et al., 2005; Heidemeyer et al., 2014). Six of the seven species of sea turtle are now protected under the Indian Ocean South East Asian (IOSEA) Marine Turtle memorandum of understanding (MoU). These include the; loggerhead (Caretta caretta), hawksbill (Eretmochelys imbricata), green (Chelonia mydas), leatherback (Dermochelys coriacea), flatback (Natator depressus) and the olive ridley (Lepidochelys olivacea). The impact, ghost gear (more specifically ghost nets) have on populations of these species is currently unknown and no regional impact assessment takes ghost gear into account which may undermine current conservation measures by signatory states. Wilcox et al., (2015) estimated that from the 8690 ghost nets collected in Northern
Australia as many as 14,600 sea turtles may have become entangled in them during the 1 year they were drifting. Similarly, Stelfox et al., (2015) reported alarming numbers of olive ridleys entangled in ghost nets in the Maldivian archipelago with as many as 12,200 olive ridley turtles entangled in only 752 ghost nets over 51 months (Stelfox et al. in press). These two studies alone highlight the urgent need for more robust analyses of ghost nets and turtle entanglement in the Indo-Pacific. Moreover, the fact that ghost nets are transboundary in nature, and therefore cross multiple political borders the need for collaboration between governments, NGOs, fisheries and IGOs is paramount if we are even to begin to tackle the issue.

**Project aim**

The ORP and secretariat of the IOSEA hope to build evidence surrounding the issue of turtle entanglement in ghost nets in the Indian Ocean and provide detailed assessments to the broader turtle community. We are confident that this ambitious proposal will encourage signatory states to submit data to the online portal, hosted by ORP. We hope to see the Indian Ocean become global leaders in collecting data on turtle entanglements in ghost nets and become the global model for others to follow.

**Methods to achieve the above aim**

The secretariat of the IOSEA and the Olive Ridley Project (ORP) would like to quantify the impact of ghost nets to sea turtles in the entire Indian Ocean and urge signatory states to join this initiative to build evidence on the issue of ghost net and turtle entanglements in the region.

Currently the ORP have an online platform (https://oliveridleyproject.org/report-a-ghost-net) that collects data on ghost nets and ghost net/turtle entanglements since 2013. They have one of the largest and most comprehensive databases in the Indian Ocean and use this data to help build evidence on the issue in the Maldives. They collect data through citizen scientists that
report ghost nets and turtle entanglements in the Maldives and more recently have expanded into Kenya, Oman and Pakistan. Through this data collection the ORP have been able to develop models that can predict the threat different net types face to sea turtles (Stelfox et al., in press) and broadly classify nets to fisheries according to net specifications (stelfox et al., in press). However, more data from the wider Indian Ocean will help improve model estimates. ORPs founder and CEO, Martin Stelfox developed the data collection protocol alongside the IUCN and was the coordinator for the build evidence working group for Global Ghost Gear Initiative (GGGI), a global sectoral alliance set up to tackle ghost gear globally and was a major contributor to developing the global app on ghost gear.

Here, we propose to increase ORPs efforts in ghost net recording and entanglement of turtles. Data will be utilised to build upon existing predictive models that are able to identify problematic fisheries through net identification (Stelfox et al., in press). Moreover, we hope to map the spatial distribution of ghost nets and turtle entanglements to provide evidence to the wider turtle community to incorporate into conservation management plans.

**What is needed?**

The secretariat of the IOSEA, in collaboration with the ORP would like to invite IOSEA signatory states, experts and observers to submit data on ghost nets and ghost net turtle entanglement to the online data portal ([https://oliveridleyproject.org/report-a-ghost-net](https://oliveridleyproject.org/report-a-ghost-net)). This collaboration requires no monetary signup or sponsorship and can be implemented by citizen scientists and/or scientists alike.

The database will be maintained by the ORP team and ORP will provide yearly analyses that will be presented on the CMS website. Due to the sensitive nature of ghost gear all submissions will be confidential and no publication of results will be conducted without prior permission.
of the data submitters. Furthermore, the raw data will be available to all data analysts and scientists upon request.

**Appendix 5.1** Joint proposal by the Olive Ridley Project and the IOSEA secretariat to encourage data submission from 35 signatory states of the Indian Ocean. To be placed under item 10.5, Other Opportunities for Collaboration at the 8th Meeting of the Signatories to the IOSEA Marine Turtle MOU.
Appendix 5.2 Illustrations from the childrens book I co-authored called “The Adventures of Olive the Sea Turtle”. Educational childrens book following the adventures of olive the sea turtle. Illustrations by Kelsey Dutton