

Investigating plastic ingestion and entanglement within marine vertebrates in the NE Atlantic to inform policy

Tese de Doutoramento

Yasmina Rodríguez

Doutoramento em

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*“It is a curious situation that the sea,
from which life first arose
should now be threatened by the activities of one form of that life.
But the sea, though changed in a sinister way,
will continue to exist; the threat is rather to life itself.”*
— Rachel Carson, *The Sea Around Us* (1951)

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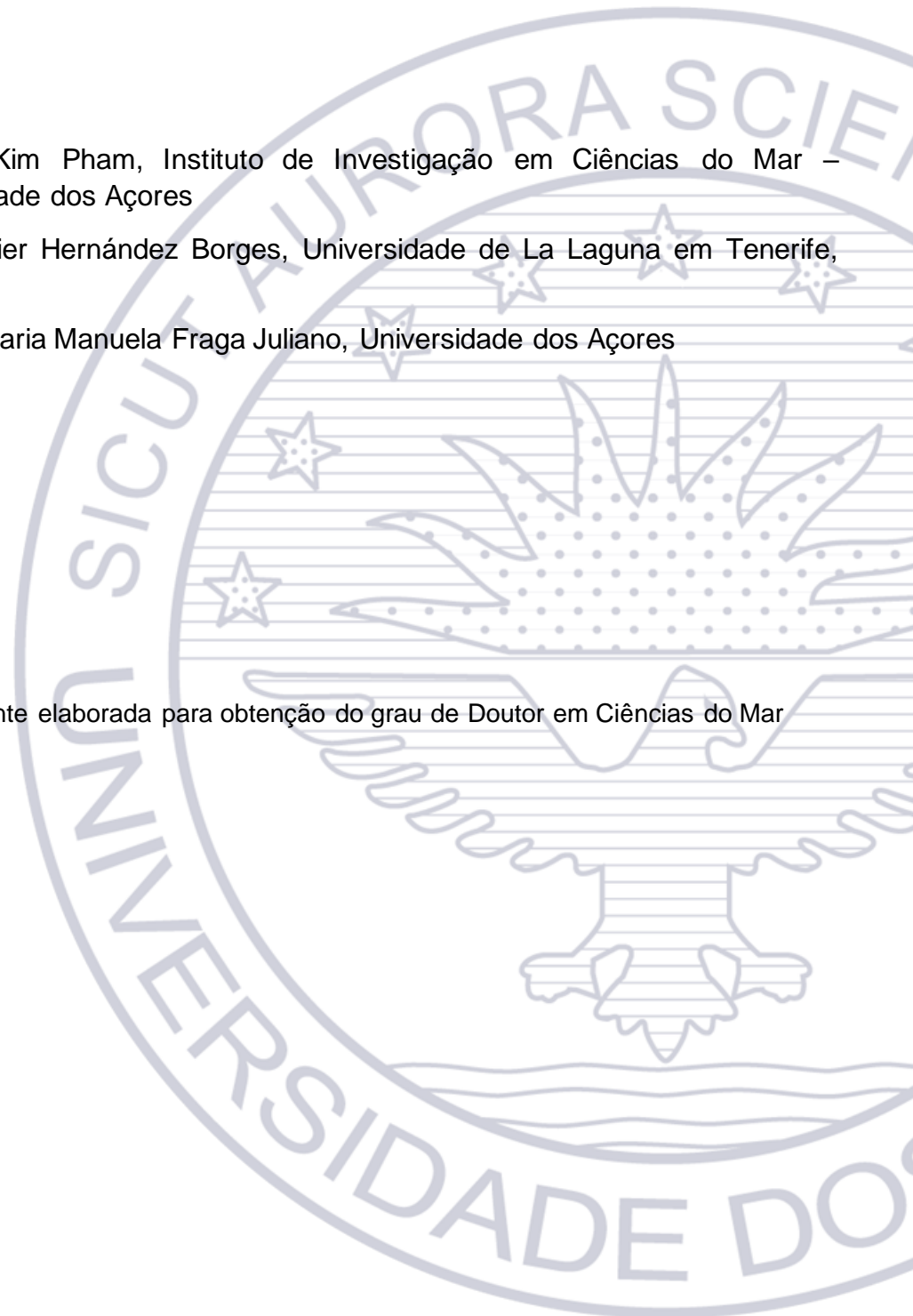
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CONTENTS

List of acronyms.....	V
Abstract.....	VI
Resumo.....	VIII
CHAPTER I	1
General Introduction	1
1.1 The widespread problem of plastic production.....	1
1.1.1. History and current status of the industry.....	1
1.1.2. Plastic manufacturing: Polymer types, main uses, and chemicals associated	2
1.2. Marine plastic pollution	4
1.2.1. The global scale of plastic in the oceans	4
1.2.2. Plastics in the Azores: Insights beyond the chapters of this thesis.....	6
1.3. The interactions between plastic and marine vertebrates.....	7
1.3.1. Plastic ingestion	7
1.3.2. Entanglement in marine litter.....	9
1.4. Monitoring, initiatives and policies for tackling plastic pollution.....	9
1.5. Goal and thesis outline.....	12
CHAPTER II	15
Cory's shearwater as a key bioindicator for monitoring floating plastics	15
Abstract.....	15
1. Introduction.....	16
2. Material and methods.....	18
2.1. Sample collection.....	18
2.2. Plastic characterization.....	19
2.3. Data analysis.....	20
2.3.1. General metrics.....	20
2.3.2. Selection of explanatory variables for trend analysis.....	20
2.3.3. Trend analysis.....	20
2.3.4. Threshold value and assessment method.....	21
3. Results	23
3.1. General results about plastic ingestion in Cory's shearwaters	23
3.1.1. Regional differences.....	23
3.1.2. Polymer identification	24
3.2. Temporal trends in ingested plastics.....	25
3.3. Threshold value calculation and assessment method	25
3.3.1. Metric selection.....	25
3.3.2. TV setting and assessment.....	26
4. Discussion.....	27
5. Conclusion	32
CHAPTER III	33
Plastics reset in an adult Procellariiform seabird species during the breeding season	33
Abstract.....	33
1. Introduction.....	34
2. Methods	36
2.1. Studied species.....	36
2.2. Sample collection and processing.....	37
2.3. Identification of polymers and morphologies.....	38
2.4. Data analysis.....	38
2.4.1. Trend in the number of plastics over the breeding season.....	38
2.4.2. Plastic dynamics between periods and stomach sections.....	39
3. Results	40
3.1. Trend in the number of plastics over the breeding season	40
3.2. Plastic dynamics between nesting periods and stomach sections.....	42
3.3. Polymers and morphologies.....	43
4. Discussion.....	43
5. Conclusion	48
CHAPTER IV	49
Cetaceans playing with single-use plastics (SUPs): A widespread interaction with likely severe impacts	49
Abstract.....	49
1. Introduction.....	50
2. Material and methods.....	51
2.1. Literature and social media review.....	51
2.2. Case study.....	52
3. Results	53

3.1. Literature review and social media records.....	53
3.2. Azores case study - São Miguel Island	55
3.3. General behavioural observations.....	55
4. Discussion.....	56
CHAPTER V.....	60
An index to differentiate megafauna entangled in operational fishing gears from abandoned, lost, or otherwise discarded fishing gears	60
Abstract	60
1. Introduction.....	61
2. Material and methods.....	62
2.2. Entanglement Source Assessment (ENSA) index	63
2.2.1. Approach followed.....	63
2.2.2. Criteria selection	63
2.2.3. Scoring and weights	65
2.2.4. ENSA index final classification	65
3. Results and discussion	65
3.1. ENSA index classification	65
3.2. Illustration of the ENSA index	67
3.3. Requirements for the index application.....	69
4. Conclusion	70
CHAPTER VI.....	71
Marine debris entanglements and associated impacts in megafauna across the open NE Atlantic Ocean	71
Abstract	71
1. Introduction.....	72
2. Material and methods.....	73
2.1. Study area.....	73
2.2. Data collection	74
2.3. Data categorization.....	76
2.3.1. ALDFG.....	76
2.3.2. Other plastics	76
2.3.3. Colour classification.....	76
2.4. Physical status of the entangled animals	77
3. Results	77
3.1. Overall results.....	77
3.2. Characterization of the entangling debris.....	78
3.3. Severity of the entanglements in marine debris	79
4. Discussion.....	81
4.1. Sea turtles	81
4.2. Marine mammals.....	83
4.3. Seabirds.....	83
4.4. Elasmobranchs and fish	84
4.5. Plastic typologies causing the entanglements	85
4.6. Importance of monitoring entanglements in marine debris	85
5. Conclusion	86
CHAPTER VII.....	88
General Discussion.....	88
7.1. Advances in the use of bioindicators for monitoring plastic pollution	89
7.2. Fulfilling gaps about entanglement and other interactions with litter.....	91
7.3. Thesis information feeding into policy	94
7.4. Future research.....	94
Final Conclusion.....	97
References.....	98
Appendix A – Supplementary Material chapter II	121
Appendix B – Supplementary Material chapter III	129
Appendix C – Supplementary Material chapter IV.....	133
Appendix D – Supplementary Material chapter V	135
Annex I - OSPAR’s Coordinated Environmental Monitoring Programme (CEMP) guidelines	138
Annex II – Student contribution to the thesis and research dissemination	153
II.1. Personal contribution to each of the scientific chapters.....	153
II.2. Publications included in the thesis	153
II.3. Conferences, meetings and other communications as first author.....	154
II.4. Other peer-reviewed papers published during the thesis.....	155
II.5. Some outreach and media coverage of the thesis	155

List of acronyms

ALDFG – Abandoned, Lost, or Otherwise Discarded Fishing Gear
AMAP – Arctic Monitoring and Assessment Programme
CEMP – Comprehensive Environmental Monitoring Programme
CI – Confidence Interval
EcoQO – Ecological Quality Objective
ECHA – European Chemicals Agency
edf – Effective Degrees of Freedom
EEZ – Exclusive Economic Zone
ENSA – Entanglement Source Assessment
EU – European Union
EU TGML – European Union Technical Group on Marine Litter
F – F-statistics
FTIR – Fourier Transform Infrared Spectroscopy
GAMMs – Generalised Additive Mixed-effects Models
GES – Good Environmental Status
GESAMP – Joint Group of Experts on the Scientific Aspects of Marine Environmental Protection
GGGI – Global Ghost Gear Initiative
HDPE – High-Density Polyethylene
ICG-ML – Intergovernmental Coordination Group on Marine Litter
INC – Intergovernmental Negotiating Committee
IUCN – International Union for Conservation of Nature
LDPE – Low-Density Polyethylene
MARPOL – International Convention for the Prevention of Pollution from Ships
MCDA – Multi-Criteria Decision Analysis
mm – Millimetres
MSFD – Marine Strategy Framework Directive
NASG – North Atlantic Subtropical Gyre
NB – Negative Binomial
NE – North-East
NW – North-West
OFG – Operational Fishing Gear
OSPAR – Oslo/Paris Convention for the Protection of the Marine Environment of the North-East Atlantic
PE – Polyethylene
PET – Polyethylene Terephthalate
PICES – North Pacific Marine Science Organization
POPs – Persistent Organic Pollutants
PP – Polypropylene
PS – Polystyrene
PVC – Polyvinyl Chloride
REACH – Registration, Evaluation, Authorisation and Restriction of Chemicals
REML – Restricted Maximum Likelihood
RSD – Relative Standard Deviation
RSE – Relative Standard Error
SD – Standard Deviation
SE – Standard Error
SUPs – Single-Use Plastics
SW – South-West
TV – Threshold Value
UN – United Nations
UNEP – United Nations Environment Programme
%FO – Percentage Frequency of Occurrence

Abstract

Marine plastic debris has become a critical environmental concern, ranking among the most pressing human challenges of the twenty-first century. Yet, current efforts to mitigate plastic pollution remain insufficient when compared to the projected global increase in plastic waste. Despite growing awareness and ongoing international political negotiations, plastics will continue to seriously threaten the oceans and biodiversity worldwide. Marine vertebrates, already subjected to numerous environmental stressors, are particularly vulnerable to plastic contamination and can serve as indicators to monitor various aspects of this threat. As such, the overarching objective of this thesis was to address gaps in our understanding of the different ways open-ocean vertebrates interact with plastic, providing insights to support a wide range of policies.

The first part of the thesis focused on a key seabird species, Cory's shearwaters (*Calonectris borealis*), and its potential as a bioindicator to monitor floating plastics in the NE Atlantic. Based on an eight-year time series encompassing 1,238 carcasses collected in the Azores and Canary Islands, we found that plastic contamination was pervasive in this species. The analysis revealed a significant temporal increase in the number of ingested items in fledglings, as well as pronounced differences between the two breeding regions, not only in the quantity of plastic but also in the types of morphologies ingested. These findings, combined with the species' ecological traits and the availability of robust samples, indicate that Cory's shearwater is a textbook example of a reliable bioindicator. A more detailed analysis of Cory's shearwater adults quantitatively demonstrated that progenitors reset their plastic loads, clearing out > 80% of the ingested items at the end of the breeding season. This reinforces the intergenerational transfer of plastics from adults to their offspring and, most importantly, underscores the value of fledglings as more reliable bioindicators than adults. As a result, future studies should account for this, as including breeders during or after chick-rearing may lead to underestimates of plastic ingestion and obscure temporal trends. Translating the main outcomes into concrete policy applications, both studies provided guidelines for the implementation of fledgling Cory's shearwaters as a common bioindicator under the Marine Strategy Framework Directive and the OSPAR Commission, including a data-driven threshold value. The oceanic EU-Macaronesian archipelagos can serve as observatories of marine litter, with fledgling Cory's shearwaters indicating temporal and spatial differences in environmental plastic contamination.

The second part of the thesis focused on investigating the process of entanglement of vertebrates in plastic debris, its sources, and potential causes. Firstly, we identified a previously poorly described behaviour in cetaceans. Based on opportunistic observations, we provide strong evidence that they engage in play behaviour with plastic litter worldwide. Odontocete species were observed actively carrying or throwing single-use plastics using their head and/or flippers in several areas across the Atlantic, Pacific, and Indian Oceans, as well as the Mediterranean and the Red Sea. Our results suggest that such behaviours may serve as a mechanism for plastic ingestion and entanglement. Secondly, although fishing-related items account for a significant

portion of entanglements in marine vertebrates, most studies fail to distinguish the source of those entrapments. These can occur either when the gear is operational or when it has been abandoned, lost, or otherwise discarded (ALDFG) in the marine environment. To address this uncertainty, we developed the Entanglement Source Assessment (ENSA) index. By analysing a set of criteria, ENSA quantitatively improves the consistency, transparency, and accuracy of the reporting, which is essential due to the differing mitigation and remediation measures necessary to tackle entanglements caused by either operational fisheries or marine debris. Finally, entanglements in marine litter, including both ALDFG and other plastics, were analysed in open ocean areas in the NE Atlantic, covering the Azores EEZ up to mainland Portugal. This threat is recognised as a severe impact of ocean plastic on marine megafauna, yet the rate of its occurrence remains poorly described due to challenges in collecting information. As a result, data was gathered from multiple stakeholders into a comprehensive entanglement in debris dataset spanning 17 years of observations. Within this thesis, we were able to document ten species affected, including sharks, sea turtles, cetaceans and seabirds. Some of the entanglements caused significant impacts by inflicting external injuries or constriction of critical body parts, leading to limb loss, body deformities, or death.

Overall, this thesis provides valuable insights into the presence of plastics in open-ocean areas, highlighting the complex environmental challenge posed by this human pressure and its significant impacts on marine vertebrates. The research conducted here presents new tools for tracking floating plastic in marine environments, spanning micro to macroplastics and covering various typologies. These advancements establish a robust scientific basis for monitoring by equipping decision-makers with the necessary resources to implement and evaluate the effectiveness of their measures. We truly expect that the work undertaken will contribute to the development of evidence-based policies and foster broader cooperation to safeguard marine ecosystems and biodiversity.

Keywords: Marine litter; Ocean debris; Microplastics; ALDFG; Marine megafauna; Bioindicators; Anthropogenic impacts; Threshold Value; Monitoring

Resumo

O plástico marinho tornou-se uma preocupação ambiental crítica, figurando entre os desafios humanos mais prementes do século XXI. No entanto, os esforços atuais para mitigar a poluição por plásticos continuam a ser insuficientes quando comparados com o aumento global previsto dos resíduos de plástico. Apesar da crescente consciencialização e das negociações políticas internacionais em curso, os plásticos continuarão a ameaçar seriamente os oceanos e a biodiversidade em todo o mundo. Os vertebrados marinhos, já sujeitos a numerosos fatores de stress ambiental, são particularmente vulneráveis à contaminação por plásticos e podem servir de indicadores para monitorizar vários aspetos desta ameaça. Como tal, o objetivo geral desta tese foi colmatar as lacunas na nossa compreensão das diferentes formas como os vertebrados pelágicos interagem com plásticos, fornecendo informações para apoiar uma vasta gama de políticas.

A primeira parte da tese centrou-se numa espécie-chave de ave marinha, o cagarro (*Calonectris borealis*), e no seu potencial como bioindicador para monitorizar plásticos flutuantes no Atlântico Nordeste. Com base numa série temporal de oito anos, abrangendo 1,238 carcaças recolhidas nos Açores e nas Ilhas Canárias, verificámos que a contaminação por plástico era generalizada nesta espécie. A análise revelou um aumento temporal significativo no número de itens ingeridos nos juvenis, bem como diferenças acentuadas entre as duas regiões de reprodução, não só na quantidade de plástico, mas também nas diferentes morfologias ingeridas. Estes resultados, combinados com as características ecológicas da espécie e a disponibilidade de amostras robustas, indicam que o cagarro é um exemplo de bioindicador fiável. Uma análise mais pormenorizada dos adultos de cagarro demonstrou quantitativamente que os progenitores reiniciam a sua carga de plástico, eliminando > 80% dos objetos ingeridos no final da época de reprodução. Este facto reforça a evidência de transferência intergeracional de plásticos dos adultos para as suas crias e, mais importante ainda, sublinha o valor dos juvenis como bioindicadores mais fiáveis do que os adultos. Consequentemente, os estudos futuros devem ter em conta este facto, uma vez que a inclusão de reprodutores durante ou após a época de alimentação das crias pode levar a subestimações da ingestão de plástico e obscurecer as tendências temporais. Traduzindo os principais resultados em aplicações políticas concretas, ambos os estudos forneceram diretrizes para a implementação dos juvenis como bioindicador comum ao abrigo da Diretiva-Quadro Estratégia Marinha e da Comissão OSPAR, incluindo um valor-limite baseado em dados. Os arquipélagos oceânicos da UE e da Macaronésia podem servir como observatórios de lixo marinho, com os cagarros a sinalizarem diferenças temporais e espaciais na contaminação ambiental por plásticos.

A segunda parte da tese centrou-se na investigação do processo de emaranhamento de vertebrados em detritos de plástico, fontes e potenciais causas. Em primeiro lugar, identificámos um comportamento dos cetáceos anteriormente pouco descrito. Com base em observações oportunistas, fornecemos fortes indícios de que estes animais se envolvem em comportamentos

lúdicos com lixo plástico em todo o mundo. Foram observadas espécies de odontocetos que transportam ou atiram ativamente plásticos de um único uso, utilizando a cabeça e/ou as barbatanas, e em várias zonas dos oceanos Atlântico, Pacífico e Índico, bem como no Mediterrâneo e no Mar Vermelho. As nossas observações sugerem que estes comportamentos podem servir de mecanismo de ingestão e emaranhamento de plástico. Em segundo lugar, embora os artigos associados à pesca representem uma parte significativa dos emaranhamentos de vertebrados marinhos, a maioria dos estudos não consegue distinguir a origem desses emaranhamentos. Estes podem ocorrer independentemente da arte de pesca estar operacional ou abandonada, perdida ou descartada (ALDFG) no meio marinho. Para fazer face a esta incerteza, desenvolvemos o índice ‘Entanglement Source Assessment’ (ENSA). Através da análise de um conjunto de critérios, o ENSA melhora quantitativamente a consistência, a transparência e a exatidão dos relatórios, o que é essencial devido às diferentes medidas de mitigação e remediação necessárias para combater o emaranhamento causado pela pesca operacional ou pelo lixo marinho. Finalmente, os emaranhamentos em lixo marinho, incluindo ALDFG e outros plásticos, foram analisados em áreas de oceano aberto no Atlântico NE, cobrindo a ZEE dos Açores até Portugal continental. Esta ameaça é reconhecida como um impacto grave do plástico oceânico na megafauna marinha, mas a sua taxa de ocorrência permanece mal descrita devido a desafios na recolha de informação. Como resultado, foram reunidos dados de várias partes interessadas num conjunto abrangente de dados sobre emaranhamento em detritos, incluindo 17 anos de observações. No âmbito desta tese, foi possível documentar dez espécies afetadas, incluindo tubarões, tartarugas marinhas, cetáceos e aves marinhas. Alguns dos emaranhamentos causaram impactos significativos ao infligir lesões externas ou constricção de partes críticas do corpo, levando à perda de membros, deformações corporais ou morte.

Globalmente, esta tese fornece informações valiosas sobre a presença de plásticos em zonas de oceano aberto, realçando o complexo desafio ambiental colocado por esta pressão humana e os seus impactos significativos nos vertebrados marinhos. A investigação aqui realizada apresenta novas ferramentas para o rastreio de plásticos flutuantes em ambientes marinhos, desde microplásticos até macroplásticos, e abrangendo várias tipologias. Estes avanços estabelecem uma base científica sólida para a monitorização, dotando os decisores dos recursos necessários para implementar e avaliar a eficácia das suas medidas. Esperamos sinceramente que o trabalho realizado contribua para o desenvolvimento de políticas baseadas em provas, e que promova uma cooperação mais alargada para salvaguardar os ecossistemas marinhos e a biodiversidade.

Palavras-chave: Lixo marinho; Lixo oceânico; Microplásticos; ALDFG; Megafauna marinha; Bioindicadores; Impactos antropogénicos; Valor limiar; Monitorização

CHAPTER I

General Introduction

1.1 The widespread problem of plastic production

1.1.1. History and current status of the industry

In a time when discovery often blossomed from pure curiosity, the experimental chemist Alexander Parkes explored endless possibilities. In 1862, amidst his alchemical explorations, he unveiled the first plastic, named Parkesine (Parkes, 1865). It was derived from nitrocellulose mixed with camphor and other additives, such as vegetable oils; a creation that may have seemed trivial, yet one that would shape the future of materials. At that time, financial and industrial interests already played a role, with many chemists and inventors seeking patents, funding, or commercial applications for their discoveries. Alexander Parkes patented Parkesine in 1855 (Parkes, 1866) and attempted to commercialise it as a substitute for ivory and tortoiseshell. However, it failed due to its poor durability and high production costs. Parkesine was later refined by John Wesley Hyatt in 1866. Hyatt developed a substance that was more flexible and durable; however, it was still highly inflammable due to the presence of nitrocellulose. Nonetheless, this gave rise to celluloid, known as the first thermoplastic, a substance of limitless potential (Rasmussen, 2021). As time wove its threads of innovation, Bakelite emerged in 1907, created by Leo Baekeland. It became the first fully synthetic plastic, or polymer, heralding a new age of materials (Feldman, 2008).

Plastics expanded beyond the military sector only after World War II, as wartime advances in polymer chemistry and abundant petroleum resources enabled industries to transition to massive production for consumer goods (Fisher, 2013). Such plastic mass manufacture and widespread use began around the 1950s, mainly based on what are known as ‘commodity plastics’, characterised by their high volume and low cost (Feldman, 2008). Surpassing the production of most other human-made materials, the subsequent increase in plastic production was remarkable. Since then, the global manufacturing growth curve has shown a consistent upward trend, with approximately 460 million metric tons (Mt) of plastic produced annually (IUCN, 2024). This level of production has reached a point where it is difficult to imagine a world without these synthetic polymers in daily life.

Since plastic production began, at least 8,300 million metric tons (Mt) of virgin plastics have been produced, with projections suggesting that only 30% of all plastics ever made are still in use (Geyer et al., 2017). Approximately 60% of all plastics manufactured have been discarded as waste, thus ending up in landfills, incinerated, or dispersed into the environment, including the

oceans (Geyer et al., 2017). Only about 10% has been recycled, often downcycled into lower-quality products (d'Ambrières, 2019). While these figures are already concerning, estimates also indicate continued exponential growth, with plastic manufacturing anticipated to reach 1 billion tonnes per year by 2050 if global primary production follows its current growth trajectory (Geyer, 2020). If production and waste management practices remain unchanged, approximately 155 to 265 Mt of mismanaged plastic waste may accumulate annually by 2060, with future loads remaining disproportionately high in Africa and Asia (Lebreton and Andrady, 2019). Yet, wealthier nations such as the United States and Europe consume significantly more plastic (~20 times up) per capita than developing countries, underscoring a significant imbalance worldwide (IEA, 2018). Indeed, toxic waste, including plastics, is exported to former colonial states through both legal agreements and illicit waste trade, in what is now referred to as waste neocolonialism (Stoett, 2024). This has become a key aspect of environmental and plastic litter justice; yet, it also provides livelihoods for millions in marginalised, peripheral economies (Stoett, 2024).

Reversing current trends of plastics entering the environment requires improved waste management, strong plastic reduction efforts, and support for industry-dependent groups to ensure global sustainability (Geyer, 2020). Yet, research on waste management has lagged behind the rapid surge in plastic production, particularly single-use plastics, outpacing infrastructure development. Moreover, China's 2017 import ban led to the collapse of the global plastic waste trade. Although major plastic waste exporters, such as Japan, the USA, Germany, and Australia could help to reduce the magnitude of the problem by expanding their treatment capacity (Li et al., 2021), current waste strategies have prioritised disposal (e.g., landfilling and incineration) over sustainable recycling and circular economy solutions. Furthermore, recycling remains inefficient due to the variety of polymers, high level of contamination in plastics, and weak economic incentives (Singh and Walker, 2024).

1.1.2. Plastic manufacturing: Polymer types, main uses, and chemicals associated

Plastic production begins with refining processes that primarily transform crude oil, natural gas, and coal into feedstocks (e.g., naphtha, ethane, propane...), which are subsequently converted into valuable chemicals known as 'petrochemicals' (Chapman, 1991). Petrochemicals are used to produce monomers, which serve as the building blocks of plastic polymers (Speight, 2019). The global market demand for plastics is primarily dominated by thermoplastics, which include polymers such as polypropylene (PP), low-density polyethylene (LDPE), high-density polyethylene (HDPE), polyvinyl chloride (PVC), polystyrene (PS), and polyethylene terephthalate (PET). These polymers are then transformed into various products. The largest segments of the global plastic market include packaging (44%), building and construction (18%), the automotive industry (8%), electrical and electronics manufacturing (7%), household, leisure,

and sports (7%), agriculture and gardening (4%), and other uses (PlasticEurope, 2022). Petrochemicals account for 12% of global oil demand, a share that is expected to grow (IEA, 2018). The plastic industry also plays a significant role in climate change, being responsible for at least, 4.5% of global greenhouse gas emissions (Cabernard et al., 2015), such as CO₂ (Stegmann et al., 2022). By 2050, this industry could consume up to 20% of global petroleum annually, while also contributing to 15% of carbon emissions (World Economic Forum, 2016). In the European Union (EU), plastic production generates approximately 13.4 Mt of CO₂ per year, accounting for around 20% of the total emissions from the chemical industry within the EU (Mortensen et al., 2021). In general, because plastics are produced primarily from fossil fuels, greenhouse gas emissions occur throughout various stages of their life cycle. These stages include extraction, refining, manufacturing, product transport, waste management, and recycling processes, which all release significant amounts of CO₂ and methane, contributing to rising global temperatures (Sharma et al., 2023).

Plastics are not entirely inert, as they are made from a complex mixture of polymers and additives introduced during manufacturing processes to enhance polymer properties and prolong their lifespan (Hahladakis et al., 2018). Common additives include flame retardants, stabilisers, antioxidants, plasticisers, pigments, fillers, lubricants, acid scavengers, antistatic agents, and UV protectors. These additives provide new characteristics to the polymers, such as flexibility, durability, transparency, and colour (i.e., to make them aesthetically appealing), strength (i.e., to absorb shocks and impacts without cracking), and safety features (i.e., UV blockers) (Murphy, 2001). Additives also improve flow properties such as melting, moulding, and drying, which help to lower manufacturing costs. Despite their usefulness, additives in polymer products can contaminate soil, air, water, and food, posing potential human exposure risks via food-contact materials and during recycling or recovery processes (e.g., Wagner and Schlummer, 2020; Turner and Filella, 2021).

Some other contaminants may also be associated with plastics during manufacturing, or adsorbed (thus, stick to the surface) once plastics enter the environment (Yu et al., 2019). In Europe, the use of additives in plastics is regulated through limitations and bans under the REACH (Registration, Evaluation, Authorisation, and Restriction of Chemicals) regulation (EC 1907/2006). REACH holds the industry responsible for managing chemical risks and providing safety information by requiring manufacturers and importers to collect data on their substances' properties and register them in the European Chemicals Agency (ECHA) database. Yet, despite these restrictions, hazardous additives, including those that contain trace metals (e.g., Al, Cu, Zn, Pb, and Ag) and persistent organic pollutants (POPs, e.g., PCBs, PAHs, and DDT), persist in

plastics in circulation due to the widespread use of many products and broader contamination of recycled materials (Conti et al., 2021).

Heavy metals (and metalloids) have been widely used in the industry as metal-based additives, for example, as fillers in plastic composites (e.g., Adeniyi and Ighalo, 2021). Additionally, once in the ocean, macro- and microplastics can adsorb and desorb other trace metals, acting as a point source of these chemicals in the environment (Munier and Bendell, 2018). Yet, the primary health and environmental concern is metal diffusion from the plastic matrix, with historical metal additives posing a greater risk than metals absorbed from the environment (Turner and Filella, 2021). Other types of contaminants related to plastics are POPs. Those traditionally used as plastic additives have been linked to endocrine disruption, reproductive disorders, and various cancers. Thus, they have been listed under the global treaty of the 2001 Stockholm Convention that aims to protect health and the environment from harmful chemicals (Lallas, 2001). However, POP-containing additives in plastics continue to pose a global challenge. This issue disproportionately affects low-income countries in Africa. Many end-of-life plastic products are imported and accumulated over decades on the continent, despite limited monitoring capacity and inadequate waste management infrastructure in some countries, while the presence of POPs further undermines their recyclability (Babayemi et al., 2025). Plastics can also absorb POPs from the environment and act as vectors of contaminants to biota through plastic ingestion. Early laboratory studies suggest that microplastics can transfer sorbed chemicals to organisms at high concentrations, primarily due to the role of gut surfactants or the influence of pH or temperature on desorption, resulting in biological effects (Browne et al., 2013; Rochman et al., 2013; Wright et al., 2013). However, experimental plastic concentrations often exceed environmental levels. Some authors have argued that several studies fail to meet the standards required to support their conclusions (Koelmans et al., 2022), while others have provided evidence that the vector effect is of limited importance and therefore unlikely to influence chemical risks (e.g., Bakir et al., 2016).

1.2. Marine plastic pollution

1.2.1. The global scale of plastic in the oceans

Research on marine plastic debris dates back to the 1970s; however, it was not until 2004 that microplastics were formally documented in the marine environment (Thompson et al., 2004). Marine plastic pollution has become a pressing global environmental concern, with current estimates suggesting that by 2030, 90 Mt of plastic could enter aquatic ecosystems annually if society fails to uphold ambitious governmental commitments and transform the global plastics economy (Borrelle et al., 2020).

A variety of sources contribute to marine plastic contamination, including maritime operations (e.g., commercial and recreational fishing, and shipping) and land-based activities such as

improper waste management and industrial leakages. The export of plastic waste to lower-income countries with limited resources and inadequate waste management procedures has turned rivers into major pathways for plastic litter to reach the oceans (e.g., Lebreton and Andrady, 2019; Bishop et al., 2020; Meijer et al., 2021). Once at sea, plastics are carried by oceanic currents, becoming a highly dynamic contaminant (Maximenko et al., 2012). Ocean currents play a central role in transporting plastics to convergence zones in the open ocean, making them omnipresent and highly concentrated within the five subtropical gyres (e.g., Law et al., 2010; Cózar et al., 2014). Consequently, the global abundance of floating plastics has increased (Ryan and Moloney, 1993; Ostle et al., 2019), and is currently estimated at 82–358 trillion particles, weighing 1.1–4.9 million tons in ocean surface layers (Eriksen et al., 2023). Although concentrations showed no clear trend until 1990, they have risen rapidly since 2005 (Eriksen et al., 2023).

Floating plastics can reach other environmental compartments through various mechanisms. For example, shore deposition in coastal regions of oceanic islands has transformed remote and pristine locations into significant plastic hotspots, including both macro- and microplastics (e.g., Álvarez-Hernández et al., 2019; Pham et al., 2020; Jones et al., 2022; Shankar et al., 2023). Plastic fragmentation, and consequently, its ingestion by migratory organisms, has made certain biota a vector for the transport of plastics across distant regions (e.g., Provencher et al., 2018; Grant et al., 2021). In addition, biofouling increases the weight of plastics, allowing them to move through the water column and even reach the deep sea (e.g., Cózar et al., 2014; Porter et al., 2018). Fisheries have also become a major source of seafloor litter through abandoned, lost, or discarded fishing gear (e.g., Duncan et al., 2023; Liu et al., 2023). Overall, plastics of all sizes are widespread on the seabed (e.g., Woodall et al., 2014; Pham et al., 2014; Zhu et al., 2024), even beyond 6,000 m deep, where 92% are single-use plastics found as deep as 10,898 m in the world's deepest Mariana Trench (Chiba et al., 2018).

In addition to polluting the oceans and harming marine wildlife (Agathokleous et al., 2021), plastics also pose a significant threat to the environment by disrupting carbon cycles (Zhao and Zhu, 2025; Yao et al., 2025). Due to their slow degradation, some of their carbons are trapped and sink for centuries in their structure (Shen et al., 2020). Although plastics degrade slowly, they can also break down under sunlight, and thus, release harmful gases such as methane and ethylene into the atmosphere, thereby contributing to climate change (e.g., An et al., 2024). Moreover, ocean plastics may harm marine life by disrupting carbon storage and interfering with the biological fixation of CO₂, microbial structure and communities, functional enzyme activity, and gene expression (Shen et al., 2023).

1.2.2. Plastics in the Azores: Insights beyond the chapters of this thesis

The Azores, a volcanic archipelago consisting of nine islands, is located on the edge of the North Atlantic Subtropical Gyre. Despite its remote location, far from major industries and urban centres, studies conducted over a decade have revealed significant plastic contamination in the region. Beginning with floating items on the sea surface, such as macro litter (Chambault et al., 2018), and extending to the microplastic fractions (Herrera et al., 2020), oceanographic models have provided evidence that these plastics may originate from non-local sources, primarily from the western part of the Atlantic (Cardoso and Caldeira, 2021). Floating litter may eventually wash ashore, with macroplastics accumulating in large quantities on some Azorean beaches (Pieper et al., 2015; Rios et al., 2018; Pieper et al., 2019), resulting in economic costs for local municipalities (Rodríguez et al., 2020). Similarly, sea users are affected by floating debris due to marine litter-related incidents, including propeller entanglements, water pump clogging, and collisions (Rodríguez et al., 2020). In contrast, marine debris originating locally, primarily from regional fishing activities, has been found on the seafloor of shallow waters between islands (Rodríguez and Pham, 2017), as well as in rich and unique deep-sea ecosystems of the Azores, including several seamounts (Pham et al., 2013; Duncan et al., 2023). In such environments, benthic organisms, including gorgonians, endemic cold-water corals, sponges, stony corals, black corals, scleractinians, and hydroids, are being impacted by these anthropogenic items (Pham et al., 2013; Rodríguez and Pham, 2017; Duncan et al., 2023).

In coastal environments, certain Azorean beaches act as transitional repositories for small plastic fragments (Pham et al., 2020). The annual input of these plastic items is estimated at 1.7 billion items, accumulating temporarily on the shorelines of the entire archipelago (Pham et al., 2020). These beaches also serve as sinks or reservoirs for small, fragmented plastics, with subsurface layers (extending down to one meter), accounting for 84% of the total plastic abundance on the beach (Pham et al., 2023). These plastic particles have been documented as containing high concentrations of trace elements (Ca, Mg, and Fe), likely attributed to the unique characteristics of the Azores volcanic region (Martins et al., 2020). Monitoring of small microplastics (< 1 mm) has been more limited; however, they have also been detected on Azorean beaches, with higher concentrations found in finer-grained sand (Rodrigues et al., 2024).

Additionally, several marine vertebrates have been exposed to marine litter in the Azores. Loggerhead sea turtles (*Caretta caretta*) have shown alarming rates of plastic ingestion, with 84% of the analysed individuals containing plastic items in their gastrointestinal tracts (Pham et al., 2017). This species was also the first in the region reported to suffer from entanglement in marine litter (Barreiros and Raykov, 2014). Juvenile green turtles (*Chelonia mydas*), although encountered less frequently, also face both threats, with a frequency of 86% of analysed turtles

having ingested plastics and 14% found entangled (Rodríguez Y. et al., 2022). The similarity between the plastic morphologies, sizes, and colours ingested by green turtles and those found in Azorean environments suggests that they may accidentally consume small plastic fragments while foraging (Rodríguez Y. et al., 2022). At a lower trophic level, the first assessment of anthropogenic particles in northern krill (*Meganyctiphanes norvegica*) collected in the Azores suggests that they may act as vectors, transferring microplastics into oceanic food webs (Villanova-Solano et al., 2024). Similarly, various pelagic and deep-sea fish have been documented ingesting microplastics with similar characteristics, with pelagic fish species showing significantly higher plastic concentrations than benthic species in the Azores (Pereira et al., 2020). Yet, benthic deep-sea sessile organisms are likely not immune to this contaminant either. Recently, a study on cold-water corals (*Viminella flagellum*) collected in the region found that these organisms can actively ingest and reject microplastics. Notably, microplastics were observed adhering to their surface with potentially high energetic costs (Pereira et al., 2024).

1.3. The interactions between plastic and marine vertebrates

Marine vertebrates, including fish, seabirds, and megafauna groups such as mammals, sharks, rays, and sea turtles, interact with plastics primarily through ingestion and entanglement (Nelms et al., 2023) and indirectly through behavioural changes (i.e., the incorporation of plastics into nests or play-related behaviours). To date, marine litter is known to impact 750 marine vertebrate species (Kühn and Van Franeker, 2020). Moreover, plastics can cause population-level effects that go beyond individual impacts, particularly for species already facing multiple anthropogenic pressures, such as climate change, overfishing, bycatch, hunting, disease, invasive species, and noise or light pollution (e.g., Dias et al., 2019; Nelms et al., 2021; Fuentes et al., 2023).

1.3.1. Plastic ingestion

To date, several studies have highlighted plastic ingestion across multiple levels of the marine food web, from tiny zooplankton (e.g., Cole et al., 2011; Villanova-Solano et al., 2024) to top predators (e.g., Fossi et al., 2017; Nelms et al., 2018; Gong et al., 2023). Specifically, plastic ingestion by marine vertebrates is a widespread phenomenon, with 44% of seabird species, 56.1% of cetaceans, and 100% of sea turtles already affected (Kühn and Van Franeker, 2020). Plastic ingestion may be influenced by various environmental and ecological factors. Marine vertebrates foraging in areas that overlap with marine litter hotspots (e.g., oceanic gyres, filaments, or regions adjacent to densely populated coastal areas) are more prone to this pressure (e.g., Duncan et al., 2017). For example, younger animals, such as post-hatchlings and oceanic juvenile sea turtles, are considered most at risk of plastic ingestion due to their feeding preferences and the overlap of their habitats with regions of high plastic concentration (Duncan et al., 2021). Similarly, sea turtles, sharks, and fish living in certain ocean basins with high levels of plastic pollution have

also been found to exhibit the highest frequencies of plastic ingestion (López-Martínez et al., 2020).

To explain the pathways leading to plastic ingestion, various hypotheses have been proposed, including direct processes (active and passive ingestion) and indirect ones (trophic transfer, intergenerational transfer) (Nelms et al., 2023). Some theories suggest that animals may actively ingest plastic because they mistake it for prey. For instance, some species may selectively ingest certain items because of visual cues, particularly if those plastics have a morphology or colour that resembles their typical food items (e.g., Schuyler et al., 2012; Duncan et al., 2019; Noh et al., 2024). Similarly, sensory cues, like olfactory signals, can also play a role in active plastic ingestion (Savoca et al., 2016; Pfaller et al., 2020). Plastics may also mimic the acoustic signal of certain prey, leading to ingestion through misperception during echolocation when deep-diving odontocetes are actively foraging (Merrill et al., 2024). In other cases, plastic consumption may occur passively, as plastics are inadvertently ingested when mixed with prey. This includes accidental ingestion by animals foraging in areas where litter accumulates (e.g., Di Benedetto and Awabdi, 2014; Rodríguez Y. et al., 2022), as well as ingestion through filter-feeding processes typical of some marine mammals, manta rays, and whale sharks (e.g., Germanov et al., 2019; Kahane-Rapport et al., 2022). Alternatively, indirect pathways involve trophic transfer, where microplastics pass from prey to predators when the latter consume organisms that have already ingested those items (e.g., Nelms et al., 2018; Miller et al., 2020). Moreover, some seabird chicks ingest plastics indirectly through intergenerational transfer, a process in which adult progenitors regurgitate plastics to their offspring (e.g., Ryan, 1988; Carey, 2011; van Franeker et al., 2011; Rodríguez et al., 2012a).

The ingestion of plastics is not only problematic due to being a non-natural process; it may also have detrimental effects on the health of marine organisms. Plastics can cause visible physical harm, including internal abrasions, blockages, and reduced stomach capacity, which may lead to false satiation, starvation, weakness, and, in severe cases, perforations or ulcerations of the gastrointestinal tract (e.g., Pierce et al., 2004; Gregory, 2009; Cole et al., 2011; Auman et al., 1997). Additionally, plastics can indirectly harm organisms at the physiological level through sub-lethal effects, such as the leaching of toxic substances (e.g., additives, metals, POPs...), which can cause endocrine disruption, alter blood and cholesterol levels, impair reproductive fitness, or reduce the overall health status of the organisms (e.g., Rochman et al., 2013; Seuront, 2018; Lavers et al., 2019; Puskic et al., 2020). New pathologies, like ‘Plasticosis’, a recently discovered fibrotic disease that alters collagen levels and causes widespread scar tissue formation, with subsequent tissue damage, also affect marine animals (Charlton-Howard et al., 2023). Animal health may also be compromised by proteomic signatures of cell lysis, multiorgan failure,

and neurodegeneration associated with the consumption of plastics (de Jersey et al., 2025). When the gut microbiota is chronically exposed to plastics, this contaminant can also interfere with the host immune system functioning (Fackelmann and Sommer, 2019), reducing commensal microbiota and increasing pathogens, antibiotic-resistant, and plastic-degrading microbes (Fackelmann et al., 2023). With recent studies in human health proving data of microplastics present in the blood, tissues, placentas, brains, and many other organs (e.g., Ragusa et al., 2021; Leslie et al., 2022; Kumar et al., 2022; Nihart et al., 2025), further research is also needed at physiological level to fully understand the health impacts caused by plastic pollution on marine vertebrates.

1.3.2. Entanglement in marine litter

Entanglement in marine litter represents another major threat that has rapidly escalated into a global issue that poses severe damage to marine life, resulting in the death of many animals annually (Senko et al., 2020). To date, 27.4% of seabird species, 39.8% of cetacean species, and 100% of sea turtles have been affected by entanglement (Kühn and Van Franeker, 2020). Fishing-related items are among the most frequently reported morphologies of litter-causing entanglements, often referred to as ghost gear, or abandoned, lost, or otherwise discarded fishing gear (ALDFG), including nets and ropes (e.g., Duncan et al., 2017; Ryan, 2018; Nelms et al., 2021). Other common entangling items include single-use plastics such as packaging straps, rings, plastic bags, and six-pack rings (e.g., Parton et al., 2019). These materials compromise animal welfare by restricting movement and causing severe injuries, which may even lead to death (e.g., Butterworth, 2016; Noh et al., 2025). Entangled animals often experience severe stress, drowning, or starvation due to impaired ability to swim, feed, or evade predators. Furthermore, other interactions between marine vertebrates and plastic litter are significant, as they may modify animal behaviours while some act as precursors to entanglement and plastic ingestion. For example, seabirds have been observed using marine litter to build their nests (e.g., Rodríguez et al., 2013; Domínguez-Hernández et al., 2024), which in some cases has resulted in chick entanglement. Despite being a significant issue, with certain entanglement hotspots already identified and known to impact numerous species globally (Høiberg et al., 2022), entanglement in marine debris remains poorly documented in scientific literature due to the challenges of collecting data (e.g., Duncan et al., 2017; Claro et al., 2019).

1.4. Monitoring, initiatives and policies for tackling plastic pollution

Introduced in 2009, the planetary boundaries concept defines the environmental limits within which humanity can still operate safely, based on measured levels of human-induced perturbation (Rockström et al., 2009). Among the nine recognised planetary boundaries, the novel entities include plastic pollution (Steffen et al., 2015). Some years ago, it remained uncertain whether the

concentration of plastic in the ocean would surpass a threshold capable of triggering global impacts on vital Earth system processes (Villarrubia-Gómez et al., 2018). Today, the rapid production and release of novel entities into the environment have pushed humanity beyond a critical threshold, positioning plastic contamination as a major concern that influences global sustainability and policy development (Persson et al., 2022; Richardson et al., 2023).

Monitoring is an effective approach to gathering data on contaminants as dynamic as plastics. It helps generate relevant results suitable for reporting and informing policy and decision-making processes (GESAMP, 2019). Monitoring involves repeated, long-term measurements of plastics in the environment and biota. It allows researchers to identify trends over space and time, using consistent sampling methodologies, while accounting for ecological and biological factors that may influence the results (GESAMP, 2019). Clear stakeholder dialogue is essential to standardise global plastic pollution monitoring, identify research priorities, and support policymakers and regulators (Lusher and Primpke, 2023). By emphasising the ecological, economic, and ethical dimensions of marine litter, global reporting can drive stakeholder engagement and support the implementation of measures aimed at reducing plastic production, improving waste management, and promoting circular economies.

Global regulations to address plastic pollution began with the London Convention, which aimed to regulate the dumping of harmful materials into the ocean. This was later upgraded due to the London Protocol (1996), which strengthened these measures with more stringent restrictions on the waste types, including plastics. Similarly, MARPOL (International Convention for the Prevention of Pollution from Ships) includes Annex V, which prohibits the discharge of plastics from ships into the ocean (e.g., Chen, 2015; Serra-Gonçalves et al., 2023). Other important initiatives outside formal policy agreements, and thus, with no political commitment, include the Global Ghost Gear Initiative (GGGI), an international alliance dedicated to addressing the issue of lost fishing gear (Richardson et al., 2019). Indirect initiatives include the Basel Convention, which has a program to control transboundary movements of hazardous wastes and their disposal, including plastics (Raubenheimer and McIlgorm, 2018). Similarly, the Bamako Convention bans the import of hazardous waste in Africa, including certain plastics, particularly as the continent is becoming a global hotspot for this type of pollution (Manyara et al., 2023).

The United Nations (UN) Sustainable Development Goals include Goal 4, which focuses on addressing plastic pollution through the target: *'Deal with legacy - Managing plastic waste that cannot be reused or recycled in an environmentally sound manner'*. Specifically, the UN Environment Program (UNEP) adopted in 2022 a landmark resolution to develop a global plastics treaty as a legally binding agreement aimed at reducing environmental harm, including ocean pollution and microplastics. It is expected that this treaty caps plastic production, thereby reducing

it at the source through preventative measures (e.g., Bergmann et al., 2022; Landrigan et al., 2023). It primarily focuses on addressing the entire life cycle of plastics (e.g., substitution, improved recycling and waste management, circularity...), including chemical regulation. Over 16,000 different chemical substances may be used in plastic production, more than 4,000 of which are hazardous to humans and the environment, yet only 6% are currently regulated (Wagner et al., 2024; Brander et al., 2024). Therefore, a plastic treaty should also aim to simplify the chemical composition of plastics, ensure transparency in chemical usage, and promote incentives for a well-structured transition (Wang and Praetorius, 2022). Plastic pollution disproportionately harms vulnerable populations, particularly in developing countries, making it essential to uphold procedural environmental justice by ensuring that Indigenous peoples and marginalised communities are included and represented in decision-making processes (Dauvergne, 2023). The first Intergovernmental Negotiating Committee (INC) meeting was held in November 2022 in Punta del Este, Uruguay. Subsequent sessions have been held in several other locations, bringing together representatives from governments, industry, academia, civil society, Indigenous Peoples, and other stakeholders. The ‘Scientist Coalition for an Effective Plastic Treaty’ has called for the establishment of a dedicated Science Policy Interface. They want to guarantee interventions and information sharing with other panels to promote a real deal to tackle plastic pollution throughout the full plastic life cycle, and with consideration for regional heterogeneity (Farrelly et al., 2024). Despite their relevance and initial prospects, the plastic treaty has limited policy advances so far (Stoett et al., 2024).

In Europe, the Marine Strategy Framework Directive (MSFD/2008/56/EC) provides the foundation for assessing and achieving Good Environmental Status (GES), including the monitoring of marine litter under Descriptor 10. As part of this effort, the Oslo/Paris Convention for the Protection of the Marine Environment of the North-East Atlantic (OSPAR, 2008) has committed to addressing pollution, emphasising the need for standardised methodologies to track marine plastics and their impacts on wildlife. However, significant information gaps remain, particularly for marine vertebrates, where data on ingestion and entanglement are crucial for regional assessments and for understanding the broader global impact of plastics on marine ecosystems. Reporting such information is crucial for informing and shaping effective policy interventions. Robust monitoring data enable the identification of marine litter hotspots, high-risk species, and the abundance, trends, and sources of pollution, which can guide targeted mitigation measures.

Specifically, bioindicator species have proven highly effective in assessing plastic levels in biota (e.g., Bonanno and Orlando-Bonaca, 2018), and, by extension, in estimating environmental plastic concentrations. However, their use remains limited, with few monitoring programs

implemented worldwide (Savoca et al., 2025). More specifically, Descriptor 10 (D10) and Criteria 3 (C3) of the MSFD require Member States to determine whether *‘the amount of litter and micro-litter ingested by marine animals is at a level that does not adversely affect the health of the species concerned. Member States shall establish threshold values for these levels through regional or subregional cooperation’* (EU 2017/848). The OSPAR Commission is another regulatory framework that utilises bioindicator species in Europe. The primary species used for this role so far is the Northern Fulmar (*Fulmarus glacialis*), which is currently used to monitor plastic pollution under the Ecological Quality Objective (EcoQO) (Van Franeker, 2011; van Franeker et al., 2021). However, this monitoring applies only to North Sea states, as the Southern European countries lie outside the distribution range of this species. Seabirds are particularly recommended as bioindicators due to the high risk of certain species ingesting plastic litter, their widespread abundance, accessibility at breeding colonies, sea-exclusive foraging habits, and extensive geographic range (e.g., Van Franeker and Law, 2015). In this sense, in 2020, data on plastic ingestion from the stomach contents of Cory’s shearwaters breeding in the Azores were included in the Portuguese MSFD D10C3 reporting. Subsequently, their potential as a bioindicator for OSPAR Region V was presented to the ICG-ML. However, the formal implementation of a long-term monitoring programme by the Azorean administration was postponed until ongoing scientific studies in the framework of the current PhD thesis were completed.

The MSFD also includes D10C4 which refers to *‘the number of individuals of each species which are adversely affected due to litter, such as by entanglement, other types of injury or mortality, or health effects. Member States shall establish threshold values for the adverse effects of litter, through regional or subregional cooperation’* (EU 2017/848). Oceanic islands like the Azores may serve as crucial locations for documenting entanglement events, providing valuable data to inform policy decisions and conservation strategies. Before the commencement of this PhD thesis, records of this type of threat were limited and largely opportunistic (Barreiros and Raykov, 2014). A previous European project, INDICIT Consortium (2018), in which we were partners, facilitated the creation of a database documenting entanglements based on events reported via social media. The second section of this PhD thesis was developed using that baseline, while also engaging directly with stakeholders.

1.5. Goal and thesis outline

Building on the background presented, the general goal of this thesis was to address key knowledge gaps and enhance our understanding of interactions between plastics and marine vertebrates, both regionally and internationally. Moreover, the thesis aims to inform policymakers by providing practical tools for monitoring plastic pollution over time. Specifically, the first part

of the thesis focuses on investigating the suitability of a seabird species as a bioindicator for monitoring floating plastics in the NE Atlantic, supporting regions that currently lack an appropriate sentinel species (Chapter II). This includes relevant information about the residence of plastics in the progenitors during the breeding season (Chapter III).

The second part of the thesis addresses additional interactions between marine megafauna and plastic litter. It begins with the first documentation of a previously unreported global behaviour between certain marine megafauna and floating plastics, including information of potential impacts derived from such interactions (Chapter V). This is followed by the development of a systematic and transparent method for distinguishing the source of entanglements in fishing-related items (Chapter VI). Finally, opportunistic data are used to present an overview of marine litter entanglements and their impacts across various megafauna groups, establishing the first baseline of marine litter entanglements in the open ocean waters of the NE Atlantic (Chapter VI).

Each research chapter is summarised as follows:

Chapter II: Plastic ingestion was investigated in 1,238 deceased Cory's shearwaters (*Calonectris borealis*) to assess their potential as bioindicators of plastic litter. The data was collected over eight years from breeding areas of the Macaronesia region, including the Azores and the Canary Islands, and covering two age groups (adults and fledglings). This bioindicator's essential parameters were defined through spatiotemporal analyses. The target age group, sampling approach, and pollution threshold value were also established. This chapter's final aim was to support the official recognition of Cory's shearwaters as a floating plastic bioindicator within the frameworks of the MSFD and the OSPAR Commission.

Chapter III: Plastic ingestion was investigated in adult Cory's shearwaters over the breeding season to examine an understudied mechanism related to the retention of plastics in progenitors' seabirds. Different nesting stages (pre-laying, incubation, and chick-rearing) were analysed to compare plastic loads and sizes between stomach sections (proventriculus and gizzard). The overall aim was to determine whether plastic concentrations decrease as a result of intergenerational transfer from breeders to offspring, including the extent of this transmission. Furthermore, this chapter explores the policy implications of using adult breeders as bioindicators.

Chapter IV: This chapter explores interactions between cetaceans and plastic litter, highlighting a behaviour that is underreported in the scientific literature, in which several odontocete species engage in play with plastic debris. The main dataset was obtained from a whale-watching operator in the Azores Archipelago that provided photographs documenting cetacean play behaviour dating back to 2011. Additionally, an extensive review of scientific literature was conducted using Google Scholar and Web of Science, and complemented by a thorough analysis of online media platforms to identify global records of cetaceans interacting with plastic waste.

Chapter V: To minimise uncertainty when determining whether an entanglement was caused by an operational fishing gear (OFG) or by an abandoned, lost, or otherwise discarded fishing gear (ALDFG), this thesis developed the Entanglement Source Assessment (ENSA) index. This index systematically evaluates entanglement cases by applying pre-defined criteria related to both the entangling item and the affected organism, using a Multi-Criteria Decision Analysis (MCDA).

Chapter VI: This chapter investigates entanglements in marine litter occurring from the Azores EEZ up to Portugal's mainland (NE Atlantic), using data collected opportunistically from two distinct sources of information (the regional stranding network and sea users, including tourist operators, researchers, fishery observers, and recreational fishers) over 17 years. Out of 120 entanglement events initially compiled, 41 had adequate visual material and were classified as caused by marine litter. These events were further analysed to determine the frequency of this threat to marine megafauna inhabiting the studied region, the species affected, and the potential impacts.

Four chapters of this PhD thesis have been published in peer-reviewed international scientific journals (Chapters II, III, IV, and V), while the last one is currently accepted for publication (Chapter VI). The thesis also includes a general Introduction (this chapter) and a final discussion (Chapter VII), which wraps up the key findings of this work. The references for the entire thesis are included in a dedicated section after the last chapter. Supplementary materials supporting chapters II, III, IV, and V are presented in Appendices A to D. Moreover, Annex I contain an official international policy document developed for the OSPAR Commission. This document is OSPAR's Coordinated Environmental Monitoring Programme (CEMP guidelines) and was written from some of the thesis outcomes (Chapters II and III). Finally, Annex II provides a list of additional relevant information produced during the current thesis, including other peer-reviewed publications, conference papers, policy reports, meeting attendances, media coverage, and outreach activities.

CHAPTER II

Cory's shearwater as a key bioindicator for monitoring floating plastics¹

Abstract

The potential of using organisms as bioindicators of marine litter has been an area of general interest in multiple scientific and monitoring programs across the globe. Procellariiformes seabirds are particularly vulnerable to plastic contamination, which makes them a research focus group. This study investigated plastic ingestion in deceased fledglings and adults Cory's shearwaters (*Calonectris borealis*) collected over eight years (2015 to 2022) at two Atlantic archipelagos: the Azores and the Canaries. Necropsies were carried out in a total of 1,238 individuals showing a high prevalence of plastic ingestion (90%), with approximately 80% of items recovered from the gizzard. Fledglings carried greater plastic loads compared to adults, yet plastic morphologies were similar between both age classes. The temporal analyses conducted with generalised additive mixed-effect models revealed a distinct temporal trend in plastic numbers, but not in terms of plastic mass. In addition, the spatial analyses showed that Cory's shearwaters from the Canary Islands ingest a higher quantity of plastic and a greater proportion of threadlike items than the Azorean birds. These results suggest higher contamination at the NW Africa foraging grounds next to the Canaries and highlight fisheries as a potential source of marine litter in that region. On the other hand, the information gathered from the Azorean birds suggests they would be able to monitor changes in the composition of the plastic items floating in the North Atlantic Subtropical Gyre. Overall, our outcomes support the use of Cory's shearwater fledglings that are victims of light pollution as a key bioindicator of plastic contamination in the North Atlantic. For its policy application, the presented threshold value in combination with the assessment method will enable effective tracking of floating plastic litter in the framework of the MSFD and OSPAR.

¹ Rodríguez, Y., Rodríguez, A., van Loon, W. M. G. M., Pereira, J. M., Frias, J., Duncan, E. M., Garcia, S., Herrera, L., Marqués, C., Neves, V., Domínguez-Hernández, C., Hernández-Borges, J., Rodríguez, B., & Pham, C. K. 2024. Cory's shearwater as a key bioindicator for monitoring floating plastics. *Environment International*, 186, p.108595. <https://doi.org/10.1016/j.envint.2024.108595>

1. Introduction

The relatively new concept of novel entities refers to anthropogenic substances introduced into the Earth's system in which synthetic materials such as plastics are included (Steffen et al., 2015; Richardson et al., 2023). Novel entities are an integral component of a framework comprising nine planetary boundaries that delineate a safe operational space, with crossing thresholds risking abrupt environmental changes (Rockström et al., 2009). Driven by the swift production and release of novel entities in the environment, it is considered that humanity has exceeded a border where the contamination by plastics is a significant area of concern (Persson et al., 2022). In fact, despite the international political commitments that are being negotiated to combat plastic pollution (e.g., Bergmann et al., 2022, Dauvergne, 2023), the production of this material is anticipated to persistently increase in the upcoming decades (e.g., Geyer et al., 2017). Coupled with its poorly managed disposal, this will lead to a subsequent escalation in the emission of plastic waste into the environment (Borrelle et al., 2020). Once at sea, oceanic currents play a central transport role of plastic at a broader scale, making this contaminant omnipresent and highly concentrated in convergence zones of all oceans (e.g., Law et al., 2010, Maximenko et al., 2012, Cózar et al., 2014, van Sebille et al., 2020).

Different approaches to monitoring plastic abundance in the marine environment aim to support effective mitigation and remediation measures (e.g., Rees and Pond, 1995, GESAMP, 2019, Salgado-Hernanz et al., 2021). Yet, acquiring accurate evaluations of plastic concentrations by conducting field campaigns at sea requires a significant investment of time and resources (Underwood et al., 2017). Alternatively, the use of living organisms to inform about the quality of the ecosystem, collectively known as bioindicators, can offer the most reliable and cost-effective way to study spatio-temporal fluctuations of a particular threat like plastic litter (e.g., Goodsell et al., 2009, Parmar et al., 2016). In recent years, such an approach has gained interest pointing to different animal taxa as potential sentinels for plastic contamination including sea turtles (Matiddi et al., 2019), fish (Bray et al., 2019), bivalves (Ward et al., 2019), cetaceans (Fossi et al., 2020) or jellyfish (Macali and Bergami, 2020).

The northern fulmar (*Fulmarus glacialis*) is a seabird whose ingestion of plastics has been investigated since the 1980s (Van Franeker & Meijboom, 2002). The Oslo/Paris Convention for the Protection of the Marine Environment (OSPAR) has implemented specific measures to track the abundance, trends, and composition of plastics by the long-time use of this species as a bioindicator across different countries of the North-East Atlantic (e.g., Van Franeker, 2004, Van Franeker et al., 2011, OSPAR, 2015). Additionally, the European Marine Strategy Framework Directive (MSFD) that aims to achieve a Good Environmental Status (GES) in European waters by targeting marine litter pollution in their Descriptor 10 (D10) (EC, 2008, Galgani et al., 2010, Galgani et al., 2023, EC., 2017), also uses the northern fulmar monitoring approach as an

indicator for GES (Van Franeker et al., 2021). More recently, fulmars were suggested as a means of tracking plastic pollution by the Arctic Monitoring and Assessment Programme (AMAP, 2021). Similarly, the North Pacific Marine Science Organization (PICES) is contemplating utilizing northern fulmars as a bioindicator (Savoca et al., 2022). The high significance of this species for nations located in the northern hemisphere implies that other regions outside the distribution range of northern fulmars also require a comparable indicator species (Bonanno and Orlando-Bonaca, 2018, BirdLife International, 2023).

Cory's shearwater (*Calonectris borealis*), like the northern fulmar, is a Procellariiform seabird listed as Least Concern by the International Union for Conservation of Nature (IUCN, 2023). In general, this species has a series of broad biological characteristics that align with the key features globally accepted for good bioindicators (e.g., Holt and Miller, 2010, Bonanno and Orlando-Bonaca, 2018, Fossi et al., 2018, GESAMP, 2019). They have been profoundly studied leading to a well-known ecology (e.g., Monteiro et al., 1996a, Granadeiro et al., 2006, del Hoyo et al., 2020), and there is an extensive understanding of their feeding behaviour (e.g., Paiva et al., 2010a, Neves et al., 2012, Alonso et al., 2018) including the ingestion of plastic litter (Petry et al., 2009, Colabuono et al., 2009, Rodríguez et al., 2012a, Navarro et al., 2023). Their wide-ranging foraging areas during the breeding season in the EU Macaronesia region (Magalhães et al., 2008, Ramos et al., 2013) could contribute to the ecological monitoring of the North Atlantic, as these seabirds are top predators in the marine ecosystem. Although a wide-scale assessment of Cory's shearwater suitability as a bioindicator of environmental plastic contamination has not yet been conducted (Rodríguez et al., 2012a), this species was recently proposed as an indicator for mercury (Hg) at an Atlantic basin scale (Gatt et al., 2020). Thus, Cory's shearwaters show potential as a multi-stressor bioindicator, for example, if collaborative efforts are coordinated into a concerted program (GESAMP, 2019). In 2019, Portugal proposed their use to monitor plastic pollution in the Azores as a target for the next reporting of the D10 of the MSFD (DQEM, 2020). Following this, the applicability of such species for the OSPAR Region V was shared with the Intersessional Correspondence Group on Marine Litter (ICG-ML). Nonetheless, its formal implementation as a long-term monitoring program led by the Portuguese administration has been delayed until data collection and analyses from ongoing scientific studies were completed. This research aims to fill this existing information gap.

The overarching objective of this study is to provide a detailed assessment of the suitability of Cory's shearwater as a formal bioindicator to monitor floating plastic litter. We determined plastic ingestion metrics and defined essential parameters for this bioindicator including target age class, collection methodology, sampling approach, and threshold value. We aim to provide reliable information to aid international policy action in areas of the NE Atlantic that currently lack an effective indicator species for plastic contamination in the marine environment.

2. Material and methods

2.1. Sample collection

Cory's shearwater has a wide distribution (BirdLife International, 2023, Dias et al., 2011; Fig. 2.1a) with a reproductive cycle starting when the adults arrive at breeding grounds in the North Atlantic and the Alboran Sea after their wintering migration (Fig. 2.1b). In this study, carcasses from 1,238 Cory's shearwaters were analysed over eight years from 2015 to 2022 (Table S1). This sample was composed of fledglings ($n = 1,127$; mean body mass = $725.08 \text{ g} \pm 6.83 \text{ SE}$) and adults ($n = 111$; mean body mass = $655.08 \text{ g} \pm 20.27 \text{ SE}$) from two main breeding populations of the NE Atlantic: The Azores (Portugal) and the Canary Islands (Spain) (Fig. 2.1b). Adults were obtained opportunistically when found dead on land or at sea during the entire breeding season (February-October). On the contrary, dead fledglings were collected systematically and exclusively during the fledging season, which occurs every year between October to November (Atchoi et al., 2021). Fledglings experience adverse effects when flying in the proximity of urban areas during their nocturnal maiden flights from their nests to the sea, resulting in compromised navigation and entrapment within artificially illuminated zones (Rodríguez A. et al., 2022). Although not all causes of these fallouts are yet clear, Cory's shearwaters are underground nesting seabirds where the limited exposure to ambient light in such dark-rearing habitats might influence the visual development of chicks, contributing to incidents during fledging (Atchoi et al., 2023). Therefore, light pollution makes otherwise healthy fledglings susceptible to collisions with human infrastructures, road accidents, or predation by domestic animals (Rodríguez et al., 2017a).

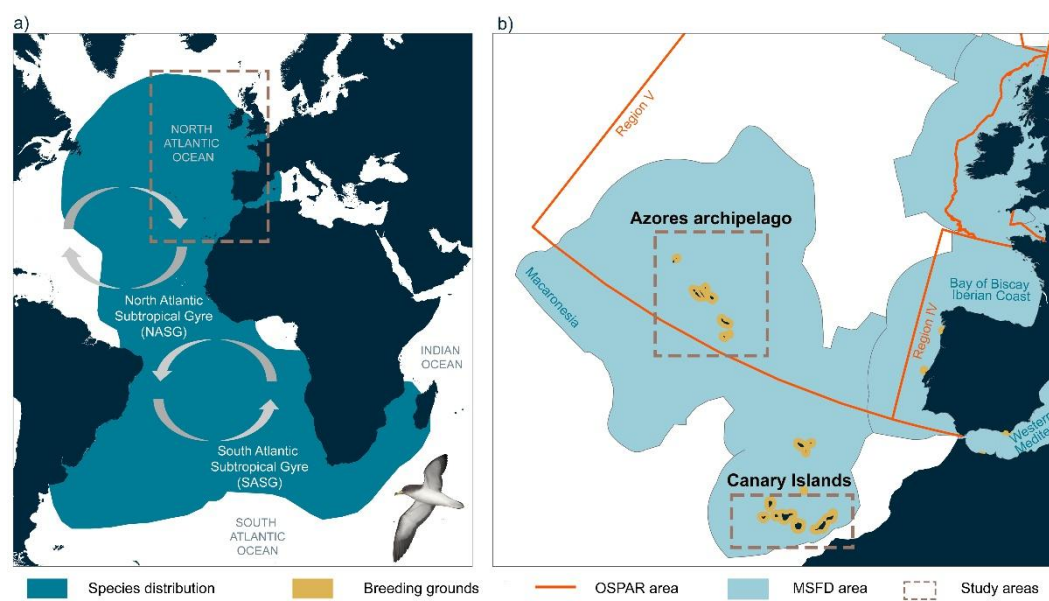


Fig. 2.1. a) Cory's shearwater distribution over the entire annual cycle including foraging trips during the breeding and the non-breeding season (Source: BirdLife International). The grey arrows represent the circulation patterns of the Atlantic Subtropical Gyres. b) Location of their breeding grounds in the North-East Atlantic and the Western Mediterranean Sea (Source: BirdLife International) identifying the two archipelagos sampled in the current study (the Azores

and the Canary Islands). The OSPAR and MSFD policy areas are delineated for all breeding regions.

Rescue campaigns for fledglings are organized by local governments and NGOs every year in the studied areas saving a high percentage of affected individuals (Atchoi et al., 2021). Yet a certain number of mortalities occur being reported to be about 6% in the Azores (Fontaine et al., 2011) and 4.8% in the Canary Islands (Rodríguez & Rodríguez, 2009). In this study, carcasses of fledglings from such casualties were collected from eight out of nine islands of the archipelago of the Azores with most samples belonging to the central island group (88%; Faial, Pico, São Jorge, and Graciosa islands), followed by the eastern islands (9%; São Miguel and Santa Maria), and finally the western group (3%; Corvo and Flores Islands). All the fledglings from the Canaries were collected on Tenerife Island.

2.2. Plastic characterization

A standardised protocol was followed during the necropsies of the dead Cory's shearwaters consisting of retrieving the entire stomach (Van Franeker, 2004, Provencher et al., 2019), and examining the contents of proventriculus and gizzard separately. All potential plastic items were recovered by filtering the contents through a 0.9 mm mesh. Plastic items were sorted from the organic matter using metal tweezers. Then, these items were cleaned in water and dried at room temperature. The total number of plastics per bird was counted and the mass was measured using a laboratory analytical balance (Mettler Toledo AB204-S) with an accuracy of ± 0.0001 g. The different plastic morphologies were classified as follows: 1) 'industrial plastic', composed exclusively of 'plastic pellets' (or nurdles), and 2) 'user plastic', which includes 'hard fragments', 'threadlike items' (remains of ropes and nets), 'sheetlike items' (e.g., pieces of plastic bags, packaging), 'foam plastics' (e.g., styrofoam or synthetic sponges) and 'others' (e.g., rubber items) (Provencher et al., 2017). Each plastic item was classified by colour into eight distinct categories: 'off/white-clear', 'black', 'blue-purple', 'green', 'grey-silver', 'orange-brown', 'red-pink', and 'yellow' (Provencher et al., 2017). Finally, each item was measured by its longest dimension to be further grouped into 'large microplastics' (1–5 mm), 'mesoplastics' (>5–25 mm), and 'macroplastics' (>25 mm) (Hanke et al., 2013, Frias and Nash, 2019, Galgani et al., 2023).

Polymer types were determined using Fourier transform infrared spectroscopy (FTIR) using a subsample from the Azorean fledglings randomly selected for the years 2016 to 2020, which comprised 12.5% of all the plastic recovered from fledglings in that region (1,384 plastic items out of 11,116). The analysed plastic items were ≥ 1 mm in size, therefore, an FTIR spectrometer Cary 630 equipped with a single reflection diamond Attenuated Total Reflectance (ATR) module (Agilent Technologies, California, USA) was used with a ZnSe beamsplitter and a 1.3 mm diameter thermoelectrically cooled deuterium triglycine sulfate (dTGS) detector. FTIR spectra were collected with 32 scans per spectrum (Happ-Genzel apodization function was applied) at a

resolution of 8 cm^{-1} in the range of 4000 and 650 cm^{-1} . Agilent MicroLab PC FTIR software was used to acquire and identify spectra using polymer libraries. The minimum matching for identification was set at $\geq 70\%$. Such criteria were set by following the indications of the Guidance of Marine Litter in European Seas of the European Commission (Galgani et al., 2023).

2.3. Data analysis

2.3.1. General metrics

Descriptive statistical results are reported in terms of plastic number, mass, size, morphology, colour, and polymer type (Provencher et al., 2017). The percentage frequency of occurrence (%FO) of plastic ingestion (Provencher et al., 2017), and the confidence limits of the %FO using the 95% Jeffrey confidence intervals (Sergeant, 2019) were calculated, including z-tests to compare sample proportions as recommended by van Franeker et al. (2021). Mean abundances for the number and mass of plastic items per bird were obtained for all individuals, including those that did not ingest plastic (Provencher et al., 2017). Arithmetic mean values (hereafter referred to as ‘mean’) are presented with the standard deviation (\pm SD) and standard error (\pm SE). To compare the ingestion of plastics between the two breeding colonies (the Azores and the Canary Islands), we used only samples that were collected in years when both archipelagos were represented. Therefore, we used the animals collected between 2015 and 2017 for these analyses (Table S2.2a).

2.3.2. Selection of explanatory variables for trend analysis

Five explanatory variables (region, age classes, year, body condition, and sex) were initially considered to assess plastic ingestion over time. To evaluate their inclusion, we explored the data by checking for outliers, collinearity, relationships, spatiotemporal patterns, zero inflation, and a balanced design of the categorical covariates following the protocol described by Zuur et al. (2010). Because the years and samples covered were not always consistent in the two regions or age classes (Table S2.2), after the exploration of the data the temporal analyses were restricted to Azorean fledglings from 2015 to 2022. This dataset involved a dependency structure since it was composed of multiple observations from each island. The sex was determined during the necropsy by visual observation of the gonads when the internal organs were not damaged (972 fledglings with sex identified out of 1,030), which allowed the inclusion in these analyses of a highly balanced sample size with similar quantities of females ($n = 480$) and males ($n = 492$). For body condition, we checked morphometric measurements with minimal missing values but we did not find any pattern with the response variables either during the data exploration or model validation, so we opted not to include any of them in the final models.

2.3.3. Trend analysis

Temporal trends were investigated by applying Generalised Additive Mixed-effect Models (GAMMs) in the two response variables selected, namely the ‘number’ (count data) and ‘mass’

(continuous data) of plastic, with the ‘mgcv’ package (Wood, 2011). This approach was followed since previous Generalised Linear Mixed-effect Models (GLMMs) were overdispersed, and a non-linear pattern effect was detected in the Pearson residuals. The two GAMMs were set with the function *gam* and a Restricted Maximum Likelihood (REML). In both models, we accounted for a temporal variation by including ‘year’ as a factor (hereafter referred to as fYear). The variable ‘island’ (factor with 8 levels, hereafter fisland) was set as a random effect to avoid pseudoreplication, whereas the variable ‘sex’ (factor with 2 levels, hereafter fsex) was included in the fixed part of the models. A Negative Binomial (NB) distribution with a log link function was selected for the GAMM applied to investigate patterns in the ‘number’ of plastics (Venables & Ripley, 2002). For the response variable ‘mass’ of plastic, a GAMM with a Tweedie distribution was the most suitable approach to deal with heterogeneity and a zero-inflation problem detected in a previous Gaussian GAMM. Since the ecological conclusions were the same as the analysis with the full dataset, an extreme observation was removed to deal with a small amount of overdispersion. The general equation applied for both models was $Y_{ij} = \text{Intercept} + f\text{Year}_{ij} + f\text{Sex}_{ij} + a_i + \varepsilon_{ij}$, where Y represents the response variables (number or mass) on fledgling i at the island j , the a_i is the random intercept for fisland which is assumed to follow $\sim N(0, \sigma_{\text{island}}^2)$ and the ε_{ij} are the residuals which are assumed to follow $\sim N(0, \sigma^2)$.

Subsequently, the model results were validated in terms of overdispersion, homogeneity, and normality by examining the scaled quantile residuals and investigating the number of zeros and the dispersion of the data by applying simulation studies following the recommendations provided by Zuur & Ieno (2016). All these assumptions were checked with the DHARMA package (Hartig, 2022). To determine the significance of the covariates, for the NB distribution we used the chi-square (Chi.sq), and for the Tweedie distribution the F-statistics (F), along with the effective degrees of freedom (edf). Relationships between the response variables were investigated using paired plots (multi-panel scatterplots) based on Pearson correlation coefficients (r). When the relevance of the results was based on p-values, a significant relationship was set at $p < 0.05$. All the statistical analyses were performed using R (R Core Team, 2021).

2.3.4. Threshold value and assessment method

A threshold value (TV) was calculated to act as the Good Environmental Status (GES) for plastic contamination using Cory’s shearwaters in the framework of OSPAR and the MSFD. The TV was computed based on the large dataset of fledglings collected in the Azores (Table 2.1; Table S2.2a). Following an in-depth baseline analysis, the adults were excluded from the TV calculations although this age class is used for other bioindicators (e.g., Van Franeker et al., 2021). The reasons for such exclusion were: 1) adults are found dead sporadically resulting in a limited number of samples per year and during specific periods, which is insufficient for monitoring

purposes (see section 2.3.2; Table S2.2b); 2) after breeding in the NE Atlantic, their transoceanic migrations to biomes associated with major upwellings in the southern hemisphere including the Benguela and Agulhas Currents, and the southern part of the Brazil Current (González-Solís et al., 2007; Fig. 2.1a) implies that plastics may have been ingested in the South Atlantic or the Indian Ocean by adults sampled at the beginning of the breeding season in the North Atlantic; and 3) the delivery of plastic to their chicks (Rodríguez et al., 2012a) implies that we would be documenting lower plastic loads in adults (Table 2.1) if they are also sampled during the chick-rearing season.

Table 2.1. Summary of the data on plastic ingestion of fledgling and adult Cory's shearwaters sampled between 2015 and 2022 in the Azores and the Canary Islands.

Region	Age classes	Sample size	%FO	Min. CI	Max. CI	Mean n° of plastic per stomach	±SE	Mean mass of plastic (g) per stomach	±SE
Azores	Fledglings	1,030	93%	91%	94%	10.8	0.4	0.0193	0.0008
	Adults	101	55%	46%	65%	2.0	0.4	0.0056	0.0013
	Total	1,131	90%	88%	91%	10.0	0.4	0.0181	0.0007
Canary Islands	Fledglings	97	99%	95%	100%	28.4	2.4	0.0258	0.0031
	Adults	10	90%	62%	99%	5.3	1.1	0.0255	0.0214
	Total	107	98%	94%	100%	26.3	2.2	0.0258	0.0031
Total	Fledglings	1,127	93%	92%	95%	12.3	0.4	0.0196	0.0008
	Adults	111	59%	49%	67%	2.3	0.4	0.0062	0.0014
	Total	1,238	90%	89%	92%	11.4	0.4	0.0185	0.0007

The decision as to which of the two parameters (the number or mass of plastics) would be the most suitable to be used for the TV calculations was primarily based on the results of the trend models (see section 2.3.3). In addition, we reinforced our decision by calculating the variability of both parameters using the relative standard deviation (RSD) and the relative standard error (RSE), with the one exhibiting the lowest RSD and RSE considered the most precise measurement. Finally, we correlated both parameters to check if one could be used as a proxy of the other (Bond & Lavers, 2023), while also checking the correlation per plastic morphology. Once the parameter was selected, the cut-off option proposed by Werner et al. (2020) was considered the most suitable approach to define the TV. In this case, it was calculated using percentile values of the selected baseline dataset. Whereas the traditional TV applied to the northern fulmar seabird bioindicator (0.1 g plastic and maximum allowed 10% exceedance) was calculated from a near-pristine area (the Canadian Arctic, Van Franeker et al., 2021), such an area

has not been identified for Cory's shearwaters. Thus, the calculations were computed by selecting a low percentile value of a large baseline dataset similar to the approach used for the beach litter compartment (*c.f.*, van Loon et al., 2020). Non-stringent values and those very close to a zero-litter option were avoided to ascertain an ambitious yet realistic proposal (Werner et al., 2020). This value was combined with the selection of a maximum exceedance percentage (%) by comparing the proposed Cory's-TV with Fulmar-TV assessment results. The selected percentile value and maximum exceedance % enabled the final definition of an optimized TV. Finally, we used a data aggregation period of five years (Kühn et al., 2023) for the assessment of the Cory's-TV. This data aggregation method provides a stable assessment value similar to the Fulmar-TV assessment method (Van Franeker et al., 2021).

3. Results

3.1. General results about plastic ingestion in Cory's shearwaters

A total of 14,133 plastic items were recovered from the stomachs of the 1,238 Cory's shearwater studied. Ninety percent (min. CI = 89%; max. CI = 92%) of all birds were affected by plastic ingestion (Table 2.1). There was a significant difference in the %FO between age classes ($z = 11.4$, $p < 0.05$), with 93% of fledglings (min. CI = 92%; max. CI = 95%) and 59% of adults (min. CI = 49%; max. CI = 67%) found with plastic items (Table 2.1).

The different age classes studied ingested similar proportions of plastic morphologies, with user plastic being the most frequent type recovered from fledglings (98%) and adults (97%), and industrial plastic representing only 2% and 3%, respectively (Table S2.3). Most of those plastic items were found in the gizzard in both age classes (fledglings = 79%; adults = 84%; Fig. S2.1). The plastic burden (number and mass of plastic) was higher in fledglings compared to adults (Table S2.2). Spatially, the overall plastic burden was also higher in birds from the Canary Islands compared to those from the Azores (Table 2.1). However, although fledglings in the Canaries ingested 2.6 times as many plastic items as Azorean fledglings, the corresponding plastic mass in the Canaries was only marginally higher (Table 2.1).

3.1.1. Regional differences

The proportion of morphologies differed between distant populations within the same years (2015-2017) (Fig. 2.2a). Fledglings from the Azores consumed more 'hard fragments' than those from the Canaries (89% vs. 39%; $z = 32.6$, $p < 0.05$; Fig. 2.2a). On the contrary, Canaries' fledglings had a notably higher percentage of 'threadlike items' compared to Azorean birds (40% vs. 7%; $z = 32.8$, $p < 0.05$; Fig. 2.2a). The remaining ingested items in the Azores included 'plastic pellets' (3 %) and 'sheetlike items' (1%), while in the Canaries there were fewer 'plastic pellets' (1%) but a noteworthy high proportion of 'sheetlike items' (20%) (Fig. 2.2a). Plastic colours were similar in fledglings for both regions (Fig. 2.2b), with 'off/white-clear' plastics being predominant in the Canaries (45%; $n = 611$ out of 1,367 items) and in the Azores (64%; $n = 1,578$

out of 2,434 items). Combining the data from the two regions, ‘off/white-clear’ items were also the dominant colour among the various morphologies (66% in ‘hard fragments’, see Fig. S2.2, 59% in ‘plastic pellets’, 48% in ‘sheetlike items’, 29% in ‘threadlike items’, see Fig. S2.3).

In general, fledglings ingested mostly ‘large microplastics’ (1–5 mm: 83%), followed by ‘mesoplastics’ (>5–25 mm: 16%) and ‘macroplastics’ (>25 mm: 1%). ‘Threadlike items’ were longer than ‘hard fragments’ largely dominated by items between 1 and 5 mm (Fig. S2.2 and S2.3). When divided by region (Table S2.4), the ‘large microplastic’ fraction clearly dominated in the Azores (92% items). The ‘large microplastics’ were also the most abundant size range in the Canaries (68% items), but their importance was combined with the ‘mesoplastics’ size fraction (31%). On average, fledglings ingested plastics that were 4.01 mm long (± 4.65 SD; ± 0.08 SE), with this length being slightly higher in the Canaries (mean = 5.59 mm) than in the Azores (mean = 3.38 mm). There were no significant differences in the average sizes of plastics ingested by fledglings for seven years in the Azores (Chi.sq = 6, df = 6, $p = 0.42$; Table S2.5).

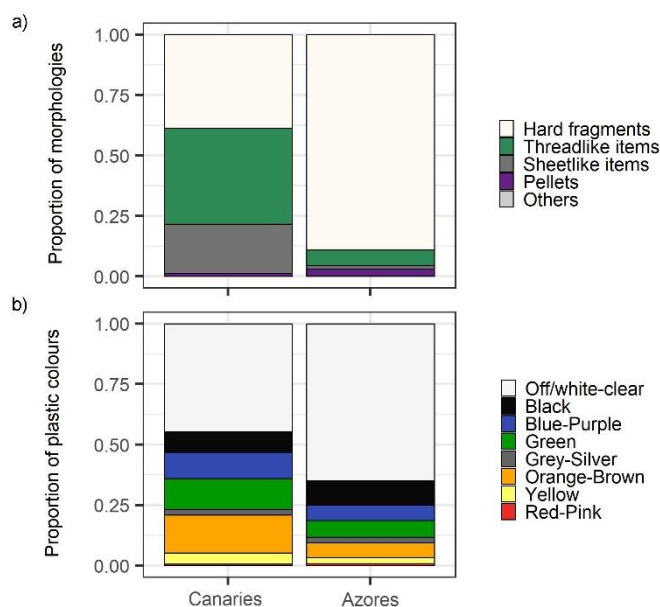


Fig. 2.2. The proportional number of a) plastic morphologies and b) plastic colours found in the stomachs of fledglings collected between 2015 and 2017 in the Azores ($n = 404$ individuals; $n = 2,434$ plastic items) and the Canary Islands ($n = 97$ individuals; $n = 1,367$ plastic items).

3.1.2. Polymer identification

The FTIR analysis showed polyethylene as the main polymer type being ingested by Azorean fledglings from 2016 to 2020 (PE: 79%; $n = 1,089$; Fig. S2.4a), followed by polypropylene (PP: 11%; $n = 148$; Fig. S2.4b). The remaining plastic items were composed of 6% poly(dimer acid-co-alkyl polyamine) (PDAPA), 3% polyamide - a high-density polymer, and 1% of other less frequent polymers (mixture of polyamide and polyester polymers = 0.29%; an epoxy resin adhesive = 0.22%; polystyrene = 0.15%; ethylene/propylene (copolymer) = 0.20%; polyacrylate

= 0.07%; and styrene-ethylene/butylene-styrene (copolymer) = 0.07%). The polymer types ingested by the species did not show variation over the period analysed (Fig. S2.5).

3.2. Temporal trends in ingested plastics

A significant positive relationship was found between the number of plastics found in Cory's shearwater stomachs from 2015 to 2022 (Fig. 2.3a), with this model explaining 27.2% of the variation in the ingested items over time (Chi.sq = 383.38, df = 7; Table S6a). On the contrary, the model applied to investigate temporal trends in the plastic mass only explained 3.52% of the variation ($F = 6.13$, df = 7; Table S2.6b), and did not show any temporal trend (Fig. 2.3b). Moreover, the 95% confidence intervals of the mass model indicate greater uncertainty in the estimates provided (Fig. 2.3b), compared to the model applied to the number of plastics (Fig. 2.3a). The sex of fledglings did not have a significant effect on plastics numbers (Chi.sq = 0.006, df = 1, $p = 0.94$) or mass ($F = 1.937$, df = 1, $p = 0.164$). Similarly, the random effect 'island' was not statistically significant for the number (edf = 2.719, Chi.sp = 5.384, $p = 0.07$; Table S2.6a) or the mass of plastics (edf = 0.023, $F = 0.003$, $p = 0.41$; Table S2.6b). The validation plots obtained during simulation studies exhibited a high level of agreement indicating that the model approaches were adequate to investigate temporal trends in both response variables (Fig. S2.6 and S2.7).

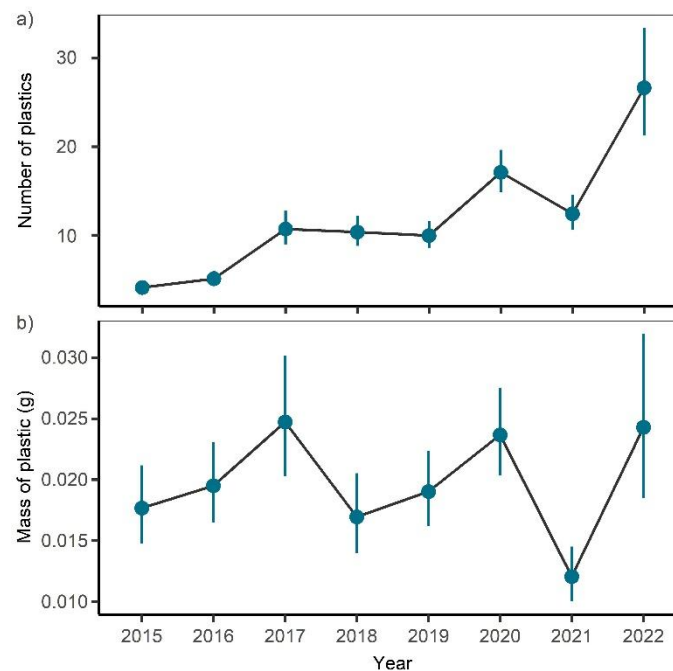


Fig. 2.3. Results of the models applied to investigate temporal trends in the numbers (a) and mass (b) of the plastic ingested by fledglings of Cory's shearwaters in the Azores from 2015 to 2022. The dots are the predicted values obtained from the GAMM models and the error bars represent the 95% confidence intervals.

3.3. Threshold value calculation and assessment method

3.3.1. Metric selection

The RSD and the RSE showed lower values for the number compared to the mass of plastics, both per island and for the total Azorean data (Fig. 2.4). In addition, the total number and mass

were correlated at 0.5 ($t = 16.46$, $df = 856$; Fig. S2.8a), indicating that when choosing one parameter for the TV, the other would be moderately represented. This, in combination with the model results (see section 3.2), show that the plastic count is preferred over the plastic mass for the TV calculations for Cory's shearwaters. When the correlation was tested separately for each plastic morphology, only 'plastic pellets' showed a strong positive relationship between their numbers and mass ($r = 0.82$, $t = 41.60$, $df = 856$; Fig. S2.8b). 'Hard fragments' ($r = 0.54$, $t = 18.90$, $df = 856$; Fig. S2.8c), 'foam plastics' ($r = 0.57$, $t = 20.11$, $df = 857$; Fig. S2.8e) and 'threadlike items' ($r = 0.51$, $t = 17.33$, $df = 856$; Fig. S2.8d) were moderately correlated. The correlation between the number and mass was very weak for 'sheetlike items' ($r = 0.21$, $t = 6.14$, $df = 856$; Fig. S2.8f).

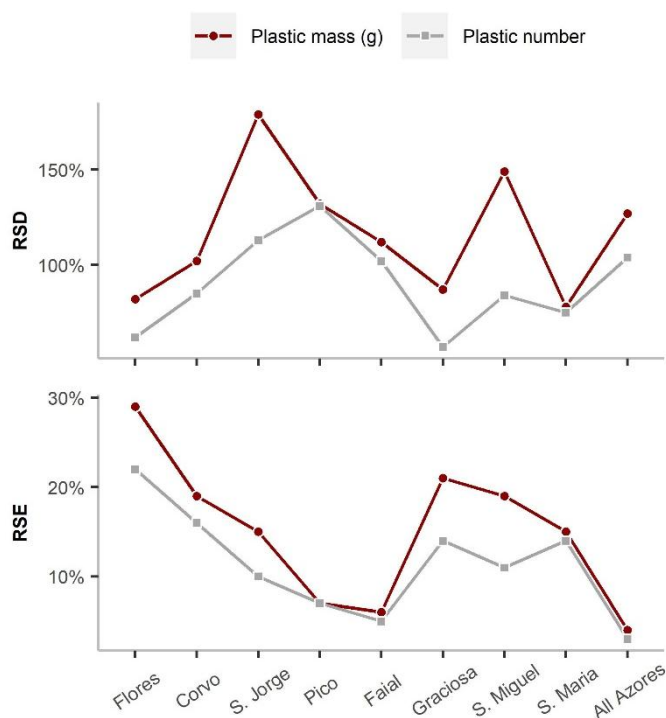


Fig. 2.4. A) The Relative Standard Deviation (RSD) and b) the Relative Standard Error (RSE) for the number of plastic items and the plastic mass (g) calculated using the entire database of fledglings collected in the Azores. The islands (x-axis) were ordered geographically based on their position in the archipelago from west to east. The variable presenting the lowest RSD and RSE values is considered the most precise measurement.

3.3.2. TV setting and assessment

The 10th, 15th and 20th percentiles were 1, 2, and 3 particles respectively (Table S2.7a). The 25th percentile corresponded to 4 plastic items (Table S2.7a), which is equivalent to 0.005 g in plastic mass (Table S2.7b). That value of 4 items coincides with the median value obtained for the island of Flores, the one where fledglings showed the lowest numbers of ingested plastics (Table S2.8). The Cory's-TV was finally set at the 25th percentile value of 4 items with a maximum exceedance of 20% (Table S2.9). Analysing the current Azores dataset, the percentage

of fledglings surpassing the limit established of 4 plastic items is consistently greater than 20% in all recent five-year periods (Fig. 2.5; Table S2.9).

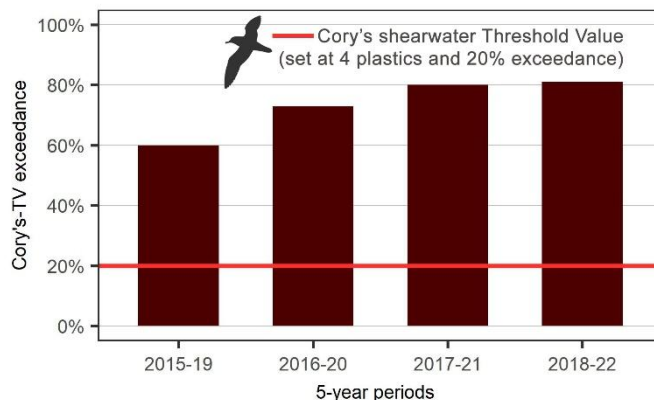


Fig. 2.5. Cory's-TV assessment of fledglings collected in the Azores over eight years. The bars represent the percentage of animals with >4 plastics in the stomachs (proventriculus and gizzards combined), aggregated and visualized for a 5-year period moving one year at a time. The horizontal line represents the Cory's-TV, which is the combination of 4 plastic items and the maximum exceedance of 20%. The Cory's-TV corresponds to 80% confidence that the Cory's-TV is reached.

4. Discussion

This study reports a high and continual prevalence of plastic ingestion (>90%) in Cory's shearwaters over eight years and across important breeding populations of the North Atlantic. The proportion of individuals found with plastics is among the highest reported in shearwater species globally (e.g., Hutton et al., 2008, Colabuono et al., 2009, Codina-García et al., 2013, Acampora et al., 2014, Roman et al., 2019a, Robuck et al., 2022), with discernible differences observed between different age classes (93%FO fledglings and 59%FO adults). This demographic distinction should be considered a relevant factor in the context of monitoring. We found that fledglings had much higher occurrence and abundance of plastics, but the same proportion of plastic morphologies compared to adults. The sample of fledglings investigated in this study had not yet self-fed, as they had succumbed to the effects of light pollution before reaching the sea. Hence, their plastic contents are the result of an intergenerational transfer during the chick-rearing period, a phenomenon already documented in this species (Rodríguez et al., 2012a) and observed in other shearwaters worldwide (Carey, 2011, Acampora et al., 2014, Rodríguez et al., 2018). Overall, this research shows the suitability of fledglings that are victims of light pollution as a bioindicator to monitor floating plastics in the marine environment. This is attributed to the several favourable characteristics associated with the ecology of Cory's shearwaters, their relationship with plastic contamination, and sample availability (Table 2.2).

Through the temporal analyses we identified a pattern exclusively within the count data and not the plastic mass. Similar to other studies on seabirds (e.g., Phillips and Waluda, 2020, Perold et al., 2020), our results indicate an increasing trend in the number of plastic items highlighting its relevance for indicating plastic abundance in the environment. This finding regarding the metric

that best works for monitoring plastic litter using Cory's shearwaters contrasts with the northern fulmar, which is being monitored based on plastic mass. Notably, although the initial Fulmar-TV was set at 10 items, it was later replaced with a 0.1 g mass equivalent (Van Franeker and Meijboom, 2002, Hamilton et al., 2023). Such redefinition occurred even when trends were observed in the number of plastics rather than in the mass (Van Franeker & Meijboom, 2002).

Table 2.2. Features that support the use of fledged Cory's shearwaters that are victims of light pollution as a robust bioindicator to monitor oceanic floating plastic items.

General characteristics of the species	Emblematic	Abundant
		Well-known ecology
		Widespread breeding distribution across coastal and oceanic locations, with high spatial coverage (near-shore and distant offshore) during foraging trips
		Public interest and awareness
Features of the target sample	Enough fledglings accidentally killed per year	Light pollution mitigation rescue campaigns facilitate the collection of a large non-invasive sample
		Plastic can be sampled through the most accurate method – necropsies - including information from proventriculus and gizzard
	Representative sample of birds	Same cohort; similar size/mass; balanced sex ratio; unbiased body condition
		A reflection of the plastic ingested by the adults over a season since these fledglings have not yet self-fed as light pollution victims usually have not reached the ocean when fatally grounded
Relation of the species with the environmental stressor	Sensitive to the disturbance	No direct mortality detected resulting from the ingestion of plastic
		Measurable response due to prevalence of plastic ingestion over time, and high %FO
		Detection of temporal trend of plastic abundance
		Correlation (indirect) with main plastic morphologies, sizes, and colours sampled in other environmental compartments

Plastic count data has been considered a less reliable metric of plastic burdens in megafauna because plastics can fragment in the digestive tracts leading to overestimation of ingested pieces (e.g., Van Franeker and Meijboom, 2002, Van Franeker and Law, 2015). However, this is still a general assumption as fragmentation of an individual item will depend on the species' gastrointestinal tract (GI) peculiarities, and the plastic characteristics including size, morphology, polymer type, weathering, and its residence time in the GI. Remarkably, a recent

study did not find evidence of a reduction in plastic size due to fragmentation in northern fulmars (Nania & Shugart, 2021), and other reported excretion of particles between 3 to 5 mm (Terepocki et al., 2017), a size range where the majority of plastic ingested by Cory's shearwaters fit. Such findings could indicate that fragmentation might not be an influential factor when considering the number of items as a metric for monitoring under certain conditions (i.e., similar species ingesting comparable plastic items), and that Procellariiform species consuming a majority of plastics within 1 to 5 mm might excrete some of those items intact. Further studies will help to comprehensively understand the dynamics of these processes.

Plastic mass is also preferred over count data as it better represents volume (Van Franeker & Meijboom, 2002; Mattidi et al., 2019), and high volume is known to potentially impact animals through obstructions or usurpation of space in the gastrointestinal tracts (Nelms et al., 2023). However, plastic mass might not be more relevant than the number of items to infer health issues. For example, in species such as Cory's shearwaters that ingest mostly small items ($\leq 5\text{mm}$), a single rounded plastic pellet could provide a high mass, whereas an irregular fragment may have a lower weight but might be more harmful due to the presence of sharp edges (Charlton-Howard et al., 2023, Nelms et al., 2023), or might carry higher quantities of pathogenic microorganisms or pollutants due to its higher surface area (Bowley et al., 2021, Yamashita et al., 2021). Recently, alongside the mass, it has been demonstrated that an increase in the number of plastic items in the guts of Cory's shearwaters and northern fulmars is linked to impacts on host resident microbiota (Fackelmann et al., 2023). Other studies have also found evidence of fibrotic diseases (Charlton-Howard et al., 2023) and increasing mortality rates (Roman et al., 2019b) associated with the number of plastic items ingested by several seabird species. Consequently, based on several previous studies, and our 8-year time series analyses where the plastic number was the metric for which trends over time were detected, we are confident in the suitability of count data for monitoring oceanic plastics using Cory's shearwaters.

We found differences in the plastic quantities and plastic morphology composition in Cory's shearwaters from distant breeding populations, which reflect variations in plastic contamination in their distinct feeding grounds (Magalhães et al., 2008, Ramos et al., 2013). Cory's shearwaters in the Canary Islands ingest an elevated proportion of 'threadlike items' (40%) compared to the Azores (7%), with the latter having a majority of 'hard fragments' (89%). This explains why we found a mean plastic mass in the Canaries similar to that in the Azores, while the number of plastic items was significantly higher in the Canarian population. This outcome is not exclusive to our results as they mirror previous studies on Cory's shearwaters in the Canaries that reported a higher number of plastics, and a high proportion of 'threadlike items' ingested by this species in that region (55% and 75%; Rodríguez et al., 2012a, Navarro et al., 2023 respectively). This also indicates that a TV based on plastic mass would be inadequate for this species as it would fail to

account for variation in the density of the plastic morphologies ingested among distant areas. ‘Threadlike items’ originate mainly from the degradation of fishing gear such as fishing lines, nets, and ropes (GESAMP, 2021). Therefore, differences in fishery operations combined with specific oceanographic features might explain this variation in plastic composition in the feeding areas of shearwaters breeding in these two different archipelagos. Indeed, Cardoso & Caldeira (2021) demonstrated that the proportion of particles present in Azorean waters are transported by the Gulf Stream, whereas the Canaries are largely exposed to NW African sources, a region that is globally considered a fishing (Stewart et al., 2010) and gear loss hotspot (Richardson et al., 2022).

Cory’s shearwaters offer several advantages over other taxa such as sea turtles, recently proposed as a bioindicator (Matiddi et al., 2017, Darmon et al., 2022). Indeed, collecting a substantial number of dead sea turtles with similar characteristics is challenging due to their diverse range of ages, size, body condition, and causes of death (Pham et al., 2017, Darmon et al., 2022). On the contrary, the number of confounding factors is far lower for fledgling shearwater victims of light pollution since artificial lights do not selectively kill birds in poor body condition (Rodríguez et al., 2012b, Rodríguez et al., 2017b, Cuesta-García et al., 2022), making them a more representative sample than, for example, beach-washed seabirds (Rodríguez et al., 2018).

Furthermore, our results demonstrated that around 80% of the plastics are found in the gizzard regardless of the age class, reinforcing necropsies as the most accurate approach for long-term assessments of plastic litter. In this sense, given that fledglings from various seabird species are globally killed by light pollution each year (Rodríguez et al., 2017a), they can serve as a reliable, cost-effective, and ethical source of samples. For example, Newell’s shearwater (*Puffinus newelli*) and the wedge-tailed shearwater (*Ardenna pacifica*) in the Pacific Ocean (Kain et al., 2016), the short-tailed shearwater (*Ardenna tenuirostris*) in the Southern Ocean (Rodríguez et al., 2018), Manx shearwater (*Puffinus puffinus*) in the Atlantic Ocean (Alley et al., 2022), or the tropical shearwater (*Puffinus bailloni*) in the western Indian Ocean (Cartraud et al., 2019), are species whose light pollution victims have been studied for plastic ingestion, with some of them being proposed as potential bioindicators. Other shearwaters affected by light pollution known to ingest plastic items are for example Scopoli's shearwater (*Calonectris diomedea*) in the Mediterranean Sea (Codina-García et al., 2013), the Cape Verde shearwater (*Calonectris edwardsii*) in the Atlantic Ocean (Matos et al., 2023), and the flesh-footed shearwater (*Ardenna carneipes*) in the Pacific Ocean which is a species with a long history of monitoring (Lavers et al., 2021). In addition, other Procellariiform families might be also affected by both, light and plastic contamination. One example is Markham's Storm Petrel (*Hydrobates markhami*), whose grounded fledglings have been also recommended as a promising indicator of marine litter for the Humboldt Current in the South Pacific Ocean (Medrano et al., 2023). The use of light pollution

victims of similar seabird species as sentinels of plastic contamination would aid comparisons and global assessments, as has been proposed for other animal groups such as mussels (GESAMP, 2019).

Bioindicators should ideally be defined through experiments that create a direct relationship between the concentrations of the stressor in the environment and the levels present in the animals (Goodsell et al., 2009). However, testing this assumption is challenging with pelagic seabirds as they predominantly fly and feed over vast oceanic areas throughout their lives (Dell'Ariccia et al., 2018). Alternatively, previous authors have used indirect measurements to determine if plastics found in seabird's stomachs reflected, or not, environmental plastics (e.g., Acampora et al., 2014, Van Franeker and Law, 2015). In the case of the Azores, the plastic items recovered from Cory's shearwaters are consistent in terms of main morphology, colour and polymer type with the ones in the same size range found on beaches (Pham et al., 2020), floating at the surface (Pham et al., 2020) or being ingested by other megafauna such as loggerhead turtles (*Caretta caretta*, Pham et al., 2017) and green turtles (*Chelonia mydas*, Rodríguez Y. et al., 2022), where 'large microplastics fragments', 'white/clear' and polyethylene items are predominant. However, in the Canaries, there is a discrepancy between the plastic morphologies commonly found on the shorelines (e.g., Álvarez-Hernández et al., 2019) or at the water sea surface (e.g., Herrera et al., 2020), and those ingested by Cory's shearwaters. This mismatch may reflect a methodological bias of field sampling of 'threadlike items'. For instance, surface tows studies available for the Canaries have been performed near the island's shoreline while the main foraging areas of Canarian Cory's shearwater breeders are on the north-western African coast, linked with the Canary Current system (Ramos et al., 2013). Notably, a high frequency of entanglements with abandoned, lost, or discarded fishing gear (ALDFG), being most likely a source of 'threadlike items', was recorded in other seabird species in the Canary Current (Rodríguez et al., 2013).

The integration of bioindicators as a crucial component in monitoring the abundance and composition of ocean plastics is required by several international political agreements (e.g., OSPAR, 2015; EC, 2017). Hence, our results provide relevant plastic litter research and monitoring information that OSPAR and the MSFD can use to cover areas outside the distribution range of northern fulmars in the North Atlantic. Cory's shearwaters will provide information where values over the TV established will trigger policy action that in turn, should result in international remediation measures (Vasilakopoulos et al., 2022). Overall, the Cory's-TV is defined as follows; "*No more than 20% of Cory's shearwater fledglings have > 4 plastic particles in their proventriculus and gizzards in a sample size of 200 birds or more, within a period of at least five consecutive years (on average 40 birds per year), collected dead per assessment area/region during the fledging season*" (Fig. 2.5). The limit value of plastics per bird was set to the 25th percentile (4 plastics) since the 10th, 15th and 20th percentiles were too close to a zero-

litter option, making those values not feasible for policy. Due to the already stringent limit value selected, a 20% exceedance limit was chosen over the 10% used for northern fulmars (e.g., Van Franeker et al., 2021; see Table S9). This 20% exceedance corresponds to an 80% confidence that the Cory's-TV is reached. The minimum sample of 40 fledglings per year was established following guidelines for monitoring plastic ingestion in seabirds (Van Franeker and Meijboom, 2002, Hanke et al., 2013, Provencher et al., 2019, Savoca et al., 2022, Galgani et al., 2023). The minimum data aggregation period selected aligns with the reporting period stipulated by OSPAR and EU TGML (Technical Group on Marine Litter; Galgani et al., 2023), yet it may be extended to six years reflecting the timeframe used for the MSFD reporting. For the Cory's-TV implementation, we emphasise the importance of also recording and reporting, when logistically possible, the mass of plastics as recommended by various policies (e.g., EC., 2017, OSPAR, 2009), the proportion of plastic morphologies to identify specific contamination sources (e.g., fishing), and the total length of each item. For simplification, automated techniques for plastic classification (size, morphology and colour) have been demonstrated to enhance data quality and precision (e.g., Lorenzo-Navarro et al., 2021, Razzell Hollis et al., 2024), making them useful for optimizing the collection of detailed data when running long-term monitoring programs.

5. Conclusion

This study demonstrates that Cory's shearwater possesses several attributes that make this species a reliable bioindicator for monitoring the relative abundance of floating plastic items in the North Atlantic. Values beyond the established threshold will serve as a stark reminder of the need to control and mitigate novel entities such as plastic litter, highlighting their significant role in environmental degradation. Long-term monitoring of fledglings would provide precise insights into the dynamics of this contaminant, ensuring the accurate monitoring of floating oceanic plastic particles in both space and time. While fledglings from the Azores would monitor changes in the composition of plastics floating in the North Atlantic Subtropical Gyre (NASG) coming mainly from the central and North American coast, those from the Canary Islands would monitor litter inputs coming from the important fishing grounds of NW Africa. In summary, fledgling victims of light pollution offer a non-invasive, readily available, cost-effective, and consistent sample of dead animals from the same cohort, making them ideal for long-term monitoring purposes. We consider that the formal implantation of Cory's shearwaters as a bioindicator in OSPAR and the MSFD will promote other shearwaters species affected by light pollution as bioindicators of plastic litter contamination across the world.

CHAPTER III

Plastics reset in an adult Procellariiform seabird species during the breeding season²

Abstract

Plastic ingestion has been extensively studied in seabirds. However, knowledge gaps remain in understanding how plastic loads behave over time and their residence inside Procellariiformes. This study investigated the temporal dynamics of ingested plastics by adult Cory's shearwaters (*Calonectris borealis*) during the breeding season to shed light on plastic retention times. We analysed the plastic content in the stomachs of 96 adults collected from 2015 to 2022 in the Azores archipelago (NE Atlantic) across distinct nesting periods: pre-laying, incubation, and chick-rearing. Our results revealed a general decrease in plastic loads over the breeding season, resulting in a marked reduction in the number of plastics before the winter pelagic phase. We found that plastic contents in breeders are mainly influenced by the intergenerational transfer of this contaminant to the offspring, indicating that progenitors highly reduce the concentrations of plastics from the stomachs (>80%) during the chick-rearing period. Understanding temporal patterns and retention time of plastic litter in seabirds is crucial for using them as indicators, with our results supporting fledglings of the studied species as the preferable age for fulfilling such a role in the breeding areas. Cory's shearwater breeders may contain plastics from their far-away wintering foraging grounds at the beginning of the breeding season, which would reflect plastics ingested in their transoceanic migrations or wintering regions beyond their breeding colonies. Moreover, they reset their plastic loads when nesting leading to underestimations if they are used as sentinels of plastic contamination during or after chick-rearing.

² Rodríguez, Y., Rodríguez, A., Pereira, J.M. and Pham, C.K., 2025. Plastics reset in an adult Procellariiform seabird species during the breeding season. *Marine Environmental Research*, 204, p.106939. <https://doi.org/10.1016/j.marenvres.2024.106939>

1. Introduction

The presence of plastic in the ocean has arisen as a critical concern (e.g., Gregory, 2009; Gall and Thomson, 2015; Thushari and Senevirathna, 2020), emerging as one of the most pressing environmental challenges of the twenty-first century (Thompson et al., 2009). Yet, efforts to mitigate plastic pollution are insufficient compared to the projected increase in plastic waste worldwide (Borrelle et al., 2020). Despite growing awareness and new instruments that are being negotiated to tackle this problem (Tessnow-von Wysocki and Le Billon, 2019; UNEP, 2023), the cumulative entry of 710 million metric tons of plastics into the terrestrial and marine environments (Lau et al., 2020) has profound and extensive ecological repercussions for ecosystems (Thushari and Senevirathna, 2020). While our comprehension of the intricate dynamics of this contaminant in biota remains incomplete, a wide range of marine organisms are known to ingest plastics globally (Kühn and Van Franeker, 2020; López-Martínez et al., 2021; Monteiro et al., 2022; Nelms et al., 2023).

Specifically, the impacts of plastic contamination on seabirds have attracted significant attention due to their far-reaching implications (e.g., Roman et al., 2019b; Fackelmann et al., 2023). In general, the Procellariiformes (albatrosses, shearwaters, petrels, fulmars, and prions) are considered among the most endangered seabird groups (Croxall et al., 2012; Dias et al., 2019), with 59% divided between near-threatened, vulnerable, endangered, and critically endangered (IUCN, 2024). Among the various human pressures affecting them, many Procellariiform species have been documented to ingest plastic litter, a threat that has been growing over time (Wilcox et al., 2015; Clark et al., 2023). To date, extensive research has provided valuable insights into this issue in many seabirds (e.g., Ryan, 1987; Auman et al., 1997; Colabuono et al., 2009; Van Franeker et al., 2011; Acampora et al., 2014; Rodríguez et al., 2018; Baak et al., 2020; Clark et al., 2023), with recent studies promoting new species as bioindicators (e.g., Cartraud et al., 2019; Franco et al., 2019; Baes et al., 2024; Rodríguez et al., 2024). However, significant gaps remain in our understanding of plastic retention times.

Based on the duration that plastic remains in the stomachs, Ryan et al. (2009) classified marine animals into three distinct groups: 1) species in which plastics are mainly regurgitated with indigestible prey remains, 2) species where the majority of plastics are eliminated through the intestines after a short time, and 3) species in which plastics are accumulated until they can be excreted. Most Procellariiformes are included in the last group due to the unique morphology of their stomachs, in which a narrow passage from the proventriculus to the ventriculus (also known as the gizzard) prevents the regurgitation of indigestible items contained in the gizzards (Furness, 1985; Roman et al., 2016). Indeed, voluntary regurgitation in these species does not empty the stomach contents, with studies using lavages or forced regurgitations indicating that expulsion of plastics occurs only from the first section known as the proventriculus (e.g., Lavers et al., 2014),

with even some plastics potentially being retained in that organ (Hutton et al., 2008). Therefore, generally in Procellariiformes, ingested plastics primarily accumulate in the gizzard (e.g., Day, 1980; Robuck et al., 2022; Rodríguez et al., 2024) which indicates that this section of the stomach may provide valuable insights into excretion rates. This is contrary to what happens in other seabird species (e.g., gulls, terns, or skuas), in which the low concentrations of plastics found during necropsies are largely attributed to the regurgitation of indigestible items from both stomach sections in the form of pellets or boluses (e.g., Acampora et al., 2017; Franco et al., 2019; Perold et al., 2024a).

Improving our knowledge of how long plastics are retained in the stomachs is relevant since seabirds acting as sentinels of marine litter contamination are essential to inform about the health of marine ecosystems (Ryan et al., 2009; Ryan, 2015; Bonanno and Orlando-Bonaca, 2018; Savoca et al., 2022). The retention time of plastics will influence whether seabirds act as a vector for the transportation of plastics between regions (e.g., Bourdages et al., 2021) or contribute to plastic fragmentation in the environment. In addition, the time that plastics reside in an organism also affects animal health through the effects of bioaccumulation and biomagnification of associated plastics' chemical contaminants (e.g., Miller et al., 2020). This includes exposure to pollutants and the risk of physical harm to different biota (Provencher et al., 2017).

In Procellariiformes, whereas some studies have reported short-term retention of plastics (Van Franeker and Law, 2015), others have suggested prolonged periods (Ryan, 2015). Such disparities and scarce research emphasise the need for a more comprehensive understanding of plastic retention times in this animal group. Yet, various confounding factors might influence the diverse conclusions drawn, complicating our understanding of the underlying processes (Day et al., 1985; Ryan, 1987). The retention of plastics in seabirds might be influenced by species-specific traits such as body size or gastrointestinal morphologies, the age of the individuals, the breeding status, and the characteristics of the anthropogenic ingested items (size, morphology, and even the chemical composition), among others (Ryan, 2015). How seabirds handle indigestible prey items might also influence plastic wear rates (and thus retention time), but so far few studies have addressed this issue (e.g., Day et al., 1985; Donnelly-Greenan et al., 2014; Van Franeker and Law, 2015; Ryan, 2016).

A crucial factor influencing plastic loads in seabirds is the transfer of plastics from parents to chicks, which has been identified in various species, including shearwaters (e.g., Carey, 2011; Rodríguez et al., 2012a), fulmars (e.g., Van Franeker and Law, 2015; Tulatz et al., 2023), and albatrosses and petrels (e.g., Young et al., 2009; Copello and Quintana, 2003; Ryan, 2015; Hyrenbach et al., 2017). This intergenerational transfer has been inferred due to the presence of plastics in nestlings or fledglings mainly when these young birds have not yet fed themselves independently. Additionally, decreases in plastic concentrations in adults during breeding seasons

have been documented in several seabird species worldwide, although with varying hypotheses to explain the declines, uncertainties as to whether the adults were breeders, or small samples in some of the studies. Evidence of decreasing plastic trends during breeding seasons has been observed in adult short-tailed shearwaters (*Puffinus tenuirostris*), cape petrels (*Daption capense*), white-faced storm petrels (*Pelagodroma marina*), and the yellow-legged gulls (*Larus michahellis*) (Skira, 1986; Van Franeker and Bell, 1988; Ryan, 2015; Nono-Almeida et al., 2023, respectively). Recently, Perold et al. (2024b) also reported that ingested plastics decreased over the breeding season in four adult seabird prey of brown skuas. Because of that, and to avoid confounding results, the authors limited their temporal assessments to the non-breeding season of those species. In northern fulmars (*Fulmarus glacialis*) and thick-billed murre (*Uria lomvia*) lower frequencies or smaller plastic concentrations have also been documented for birds later in the breeding season (Van Franeker and Meijboom, 2002; Mallory, 2008; Tulatz et al., 2023; Provencher et al., 2010).

In this research, we aimed to investigate the intricate realm of plastic temporal dynamics inside the stomachs of adult Cory's shearwaters (*Calonectris borealis*). First of all, we modelled the overall trend in plastic concentrations during the breeding season in adult breeders. Secondly, plastic concentrations were further studied in detail to a) shed light on retention times by exploring the differences in plastic loads and sizes between sections of the stomach over different nesting periods and, b) confirm whether plastic concentrations decrease due to intergenerational transfer from breeders to their offspring by also investigating the magnitude of such transmission.

2. Methods

2.1. Studied species

Cory's shearwaters are pelagic seabirds that belong to the Procellariidae family. As long-distance migrants, the members of this species travel thousands of kilometers between breeding colonies in the NE Atlantic, and non-breeding grounds in the North and South Atlantic and the Indian Ocean (e.g., González-Solís et al., 2007; Fig. S3.1). Some birds can even shift winter destinations between hemispheres and ocean basins (Dias et al., 2011). Cory's shearwaters typically nest in burrows or cavities on remote volcanic islands such as the Azores archipelago where they are the most abundant breeding seabird with more than 200,000 breeding pairs (e.g., Monteiro et al., 1996a; Furness et al., 2000). Cory's shearwaters in the Azores primarily feed on fish, such as the blue jack mackerel (*Trachurus picturatus*) and boarfish (*Capros aper*), on squids (i.e., *Neoteuthis sp.*, *Histioteuthis arcturi*, *H. reversa*, and *Taonius pavo*), and other marine organisms like crustaceans. Their foraging behaviour might vary with the availability of prey, which is influenced by oceanographic conditions (e.g., Monteiro et al., 1996b; Navarro and González-Solís, 2009; Paiva et al., 2010b; Xavier et al., 2011; Neves et al., 2012), and according to breeding duties (Navarro et al., 2007).

Cory's shearwater breeding season is divided into three periods (Monteiro et al., 1996a). The first period is known as 'pre-laying' starting when adult progenitors arrive at their breeding areas after migration in February, defend their territories, and copulate. It ends when females embark on a long trip to build up body reserves to form a single egg. The second period is 'incubation', shared by both parents that must combine fasting periods with long foraging trips (Granadeiro, 1991; Grant, 1984). The third period is 'chick-rearing' which starts after the egg hatches with breeders changing to a dual-foraging strategy (Granadeiro et al., 1998; Magalhães et al., 2008; Paiva et al., 2010a). During chick-rearing, breeders make regular foraging trips (up to 4 days) near the islands to gather food for their chicks, combined with longer trips (up to 20 days) several hundred kilometres away from the nesting site to forage for themselves on highly productive cold water upwelling areas along the mid-Atlantic ridge (Magalhães et al., 2008). During this last period, the chick is cared for by both progenitors until it fledges, normally between late October and early November.

2.2. Sample collection and processing

A total of 96 dead adult Cory's shearwaters were collected in the Azores archipelago located in the NE Atlantic (Fig. S3.1) between February and November (2015–2022) spanning various stages of the breeding season. The corpse collection was opportunistic, and for the majority, the cause of death was not registered by the collectors (60%). The remaining birds were found dead following extreme weather events such as big storms or hurricanes (25%), collisions with urban infrastructures (11%), run-overs (2%), or predation by domestic animals (1%). The birds included in this study were in apparently good condition (no diseases or starving animals documented), with the sample being divided between 32% females (body mass in g: 611.7 ± 69.8) and 68% males (body mass in g: 721.2 ± 55.1).

The stomach contents were characterized during necropsies, in which the birds were confirmed as adult breeders based on the gonad conditions and the incubation patch (Van Franeker, 2004). The stomach contents were sorted separately between the two sections (proventriculus and gizzard). After sieving the contents through a 0.9 mm mesh, all visible plastic items (≥ 1 mm) found in the stomachs were counted, weighed, and the size (maximum length in mm) of each particle was measured according to Galgani et al. (2023). The indigestible items other than plastics were also recovered from the stomachs because addressing the retention times of solid prey items might be useful in understanding the residence time of plastics inside seabirds (Donnelly-Greenan et al., 2014). Since the most common indigestible diet remains were cephalopod beaks (76% of the natural remains found in the stomachs), we focused on quantifying intact beaks or beak fragments. Overall, 84% of the studied animals contained cephalopod beaks in their stomachs, but only 49% contained both, beaks and plastic items.

2.3. Identification of polymers and morphologies

The identification of the synthetic polymers was determined for 54% of the total ingested plastic items selected randomly for the three nesting periods: 54% (n = 64 plastics) from the pre-laying, 55% (n = 33 plastics) from the incubation, and 54% (n = 13 plastics) from the chick-rearing. Fourier transform infrared spectroscopy (FTIR) was used to determine the polymeric composition of each item. The items were analysed using the PerkinElmer Frontier spectrometer with ATR attachment (universal diamond). The spectra obtained were processed with PerkinElmer's Spectrum™ 10 software and compared to nine commercially available spectral libraries of polymers provided by PerkinElmer. Only matches over 70% were considered valid identifications with most matching over 90% (Galgani et al., 2023). Additionally, plastic morphologies were classified as follows: plastic pellets, hard fragments, threadlike items (e.g., remains of ropes and nets), sheetlike items (e.g., pieces of plastic bags, packaging), foam (e.g., styrofoam or synthetic sponges) and other plastics (e.g., rubber items) (Provencher et al., 2017).

2.4. Data analysis

2.4.1. Trend in the number of plastics over the breeding season

We investigated the overall trend of plastic concentrations by modelling plastic numbers found in the stomachs of adult Cory's shearwaters over the breeding season (February–November). Data exploration was performed following the recommendations of Zuur et al. (2010) which allowed us to 1) identify the best explanatory variables and 2) detect non-linear patterns in the selected response variable 'number of plastics'. In consequence, a Generalized Additive Mixed-effect Model (GAMM) was applied with a Negative Binomial (NB) distribution and a log link function due to the discrete nature of the response variable (count data).

The NB GAMM model applied presented the following structure $n^{\circ} \text{ plastics} \sim s(\text{breeding months}) + s(n^{\circ} \text{ beaks}) + \text{sex} + \text{stomach section} + s(\text{ID}, \text{bs} = \text{"re"})$. The variable 'n° of plastics' was the total amount of plastics found per bird in each stomach section. The variable 'breeding month' was included as a smoother (s) and is denoted by the month when the animals were found dead. The variable 'number of beaks' was also added as a smoother to check the degree of association between the remains of cephalopod beaks and the plastic accumulated in the bird's stomachs. The variables 'stomach section' and 'sex' were incorporated as factors in the fixed part of the model. Since the information regarding the number of plastics was entered separately per stomach section, the 'ID' of the animals was set in the random part of the model to avoid pseudoreplication. The Restricted Maximum Likelihood (REML) was used to estimate the statistical parameters.

The model result was validated to verify that the assumptions of normality, homogeneity, and independence of covariates (testing also those not included in the model such as year and breeding period), were not violated (Zuur and Ieno, 2016). The DHARMA package (Hartig, 2022) was used

to run those validations, but also to check for zero inflation and to verify the dispersion of the data performing simulation studies. Model validations and simulation studies address a potential model misfit, patterns on the residuals, temporal correlation, or influential observations that would indicate an unreliable model result (Zuur and Ieno, 2016). Additionally, a Pearson correlation analysis was applied to determine the degree of association between the cephalopod beaks accumulated in the bird's stomachs over the breeding season. The correlation coefficient (r) and p-value were used to determine the strength and significance of the relationship between the studied variables 'number of beaks' and 'breeding months'.

2.4.2. Plastic dynamics between periods and stomach sections

We investigated the dynamics of plastic loads (numbers and mass) and sizes between stomach sections (proventriculus and gizzard), and distinct periods of the breeding season to improve our understanding of 1) the 'transfer of plastics' (referred herein to as the regurgitation of items from the proventriculus to the offspring during the chick-rearing period), and 2) the 'plastic excretion' (referred herein to the elimination of items from the gizzard to the intestines). The analysis of plastic concentrations within distinct sections of the stomachs, and across distinct stages of the breeding season, has been considered relevant to enhance the understanding and interpretation of the residence of such items inside seabirds (Provencher et al., 2017). Following this and in order to run specific statistical analyses, the data was further divided into the three nesting periods described for the studied species (Monteiro et al., 1996a): pre-laying (all the animals found dead from the beginning of the season until the middle (15th) of May; $n = 34$), incubation (animals found dead from the middle of May (16th) to the middle of July (15th); $n = 20$) and chick-rearing (adults found dead from the middle of July (16th) to the end of the season that occurs between the end of October and beginning of November; $n = 42$).

Several standard metrics were calculated including 1) the incidence of plastic ingestion known as percentage frequency of occurrence (%FO), 2) the average number and mass of plastic items known as plastic abundance (thus, using all individuals examined including the ones without plastics), and 3) the average plastic size (Provencher et al., 2017, 2019). These metrics were obtained for each nesting period and stomach section. The confidence limits for %FO were determined using the 95% Jeffrey confidence intervals (Sergeant, 2019), along with z-tests to compare sample proportions (van Franeker et al., 2011). The average values are reported with the standard error (SE) and the standard deviation (SD). Furthermore, Kruskal-Wallis non-parametric tests were used to assess differences between 1) nesting periods and, 2) the two stomach sections. Those tests were also run for the numbers, mass, and sizes of plastics. Whenever statistical significance was found in variables with many levels, the analysis was followed with pairwise comparisons by applying a Dunn post hoc test with Bonferroni correction to identify which groups differed. The statistical significance was set at a p-value <0.05 .

Finally, the decrease in plastics between nesting periods and stomach sections was calculated using fundamental arithmetic to calculate what is known as percentage of reductions. For this purpose, the following formula was applied: % of plastic reduction = $(\text{plastic numbers}_i - \text{plastic numbers}_{i+1}) / \text{plastic numbers}_i * 100$, where i denotes an initial period before the reduction, and $i+1$ represents the value of the following period after the reduction. The time between nesting periods was calculated for each of the periods defined by Monteiro et al. (1996a) by averaging the dates of deaths of our sample. In that sense, we provide a reference point to measure the length of time in days separating consecutive nesting periods based specifically on the individuals we analysed. The R Core Team (2021) version 4.1.1 was used to run all the statistical analyses.

3. Results

3.1. Trend in the number of plastics over the breeding season

A decreasing trend was found in the plastic concentrations of adult Cory's shearwaters over the months that compose the breeding season and in both stomach sections (Fig. 3.1). The NB GAMM explained 52.7% of the deviance in the data (Table 3.1). The chi-square of the variable 'breeding months' was 22.17 indicating a significant overall fit (p -value = 0.0002; Fig. 3.2a).

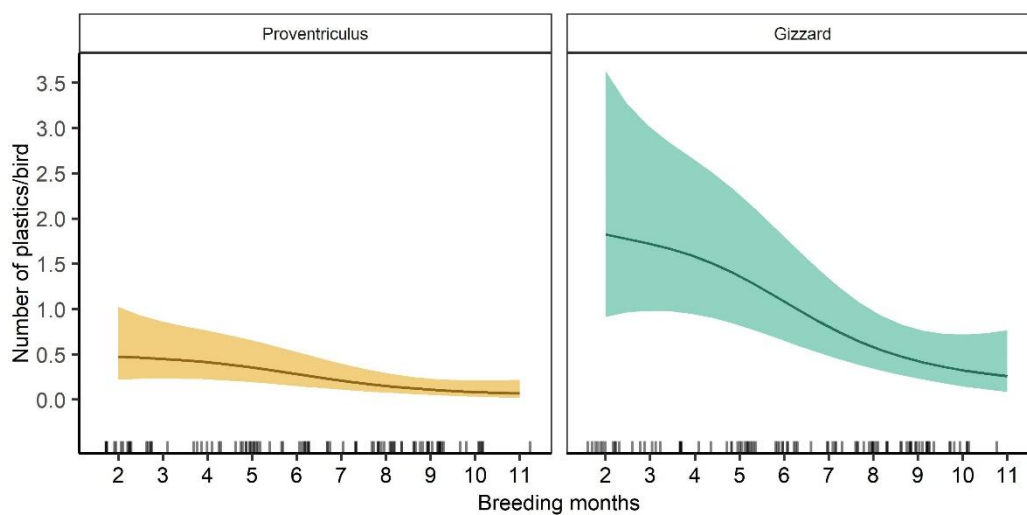


Fig. 3.1. GAMM plots for the expected number of plastics ingested by adult Cory's shearwaters over different months of a breeding season starting in February (identified in the x-axis as 2) and finishing in November (identified in the x-axis as 11) divided by the two stomach sections. The solid line represents the fitted model and the shaded area the 95% confidence intervals. The function 'geom rug' was used to plot the distribution of monthly data illustrated in the figure by the small vertical lines on the x-axis.

The smoother 'number of beaks' was statistically significant (Chi. sp = 5.88, p -value = 0.024; Table 3.1), showing an increasing trend when the number of plastics increased (Fig. 3.2b). Yet, the correlation between the number of cephalopod beaks and the breeding months was not significant at the 0.05 level ($r = 0.002$; $t = 0.02$, $df = 92$, p -value = 0.99). The random effect 'ID' was not significant in the model (Table 3.1; Fig. 3.2c) indicating no pseudoreplication in the data. The variable 'stomach section' was statistically significant (Chi. sp = 15.47, $df = 1$,

p-value <0.0001), with the gizzard holding significantly higher concentrations of plastics than the proventriculus (Fig. 3.2d). The sex was not statistically significant at the 0.05 level (Chi. sp = 0.86, df = 1, p-value = 0.35; Table 3.1), suggesting that adults of both sexes retain similar amounts of plastic during breeding (Fig. 3.2e). The statistical model demonstrated a satisfactory performance across all parameters tested including those variables not included in the model (Fig. S3.2).

Table 3.1. Summary output from the GAMM applied to investigate the number of plastics in the stomachs of adult Cory's shearwaters between different stomach sections and sexes over different months of the breeding season.

A. parametric coefficients	Estimate	Std. Error	t-value	p-value
(Intercept)	0.0953	0.3102	0.3071	0.7587
fSex (M)	-0.3212	0.3460	-0.9284	0.3532
fStomachsection (Proventriculus)	-1.3619	0.3463	-3.9325	0.0001
B. smooth terms	edf	Ref.df	F-value	p-value
s(Month_death)	1.8865	9.0000	22.1679	0.0002
s(n_beaks)	1.0361	9.0000	5.8879	0.0242
s(ID)	19.1178	94.0000	23.0506	0.0846

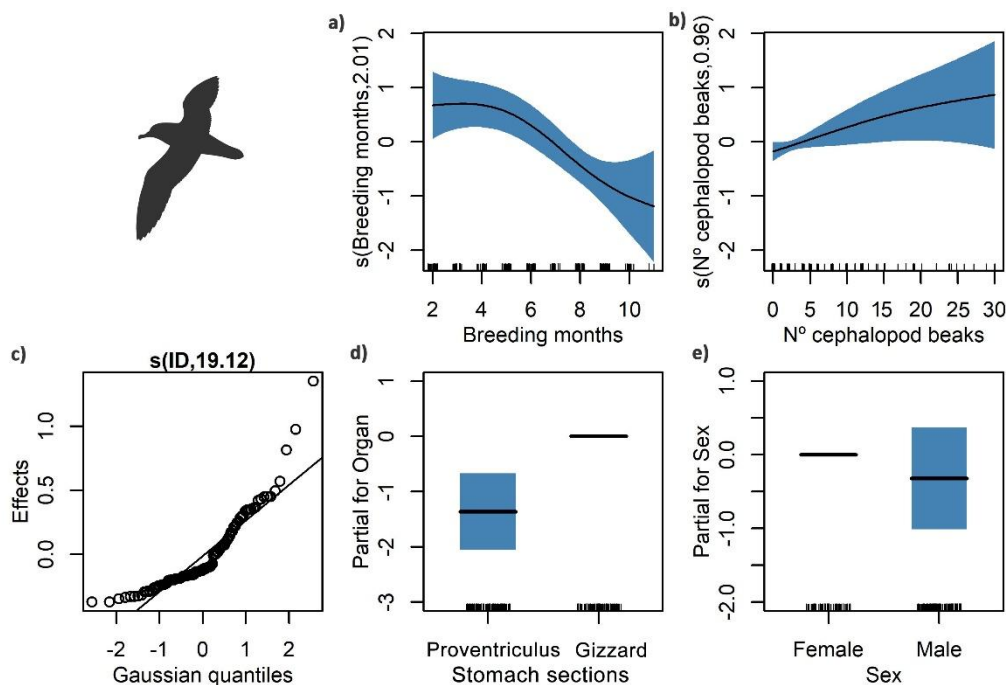


Fig. 3.2. Partial residual plots obtained from the GAMM run to investigate temporal trends in the number of plastics ingested by adult Cory's shearwaters over the breeding season. The plots are identified as follows: a) smoother breeding month starting in February (2) and ending in November (11), b) smoother of the variable number of cephalopod beaks found in the stomachs, c) random effect (ID), d) explanatory variable stomach section divided between the sections that compose the stomachs (proventriculus and gizzard), and e) explanatory variable sex with two levels (females and males). The smoothers fits (plot a and b) are shown as a solid line and the shadow areas are the 95% confidence intervals.

3.2. Plastic dynamics between nesting periods and stomach sections

The %FO of plastic ingestion was 68% (min. CI = 51%; max. CI = 81%) in the pre-laying, 75% (min. CI = 54%; max. CI = 90%) in the incubation, and 36% (min. CI = 23%; max. CI = 51%) in the chick-rearing period. %FOs were not significantly different between the two initial periods (z -value = 0.5; p -value = 0.59), but significantly lower for chick-rearing individuals compared to birds analysed from the previous period (z -value = 2.9; p -value = 0.004). The %FO of ingested plastics was different per stomach section being lower in the proventriculus compared to the gizzard in all nesting periods (see Table S3.1).

The total number of plastics for both stomach sections together was significantly different between nesting periods (Chi. sp = 18.43, df = 2, p -value <0.0001). While no differences were found in the number of plastics between pre-laying and incubation (\bar{x} = 3.5 and 3 plastics/bird respectively; see Table 3.2 and Table S3.2a), significantly lower concentrations were detected during the chick-rearing period (\bar{x} = 0.57 plastics/bird; see Table 3.2 and Table S3.2a). When the number of plastics was compared separately per stomach section, proventriculus and gizzards had significantly different plastic loads (Chi.sp = 22.10, df = 1, p -value = <0.05; Fig. S3.3). The averages were significantly lower in the proventriculus compared to the gizzard for pre-laying (Chi.sp = 14.72, df = 1, p -value = 0.0001), incubation (Chi.sp = 4.90, df = 1, p -value = 0.03) and chick-rearing (Chi.sp = 5.31, df = 1, p -value = 0.02). Specifically, the proventriculus had on average 0.59 plastics in the pre-laying, 0.4 in the incubation, and 0.1 in the chick-rearing (Table 3.2). The gizzards had on average 2.91 and 2.6 plastic items in the pre-laying and the incubation respectively, contrasting with the 0.48 plastics found during the chick-rearing period (Table 3.2).

Table 3.2. Average number and mass (including zeros), and size of plastics found in each of the two stomach sections composing seabird stomachs for the three periods that define Cory's shearwaters breeding season.

Nesting periods	Stomach sections	N° of plastics	Mean n° of plastics			Mean mass of plastics			Mean size of plastics (mm)		
			\pm SD	\pm SE	\pm SD	\pm SE	\pm SD	\pm SE			
Pre-laying (n=34 birds)	Proventriculus	20	0.59	2.11	0.36	0.0007	0.0023	0.0004	3.15	1.69	0.38
	Gizzard	99	2.91	4.23	0.73	0.0081	0.0140	0.0024	3.57	1.57	0.16
	Total	119	3.5	4.70	0.81	0.0089	0.0145	0.0025	3.75	1.15	0.24
Incubation (n=20 birds)	Proventriculus	8	0.4	0.68	0.15	0.0002	0.0007	0.0002	2.5	0.55	0.22
	Gizzard	52	2.6	4.54	1.01	0.0083	0.0185	0.0041	3.25	1.62	0.33
	Total	60	3	4.69	1.05	0.0085	0.0188	0.0042	3.22	1.03	0.30
Chick-rearing (n=42 birds)	Proventriculus	4	0.09	0.3	0.05	0.0000	0.0001	0.0000	4.75	3.1	1.55
	Gizzard	20	0.48	1.06	0.16	0.0006	0.0017	0.0003	3.95	2.48	0.59
	Total	24	0.57	1.09	0.17	0.0006	0.0018	0.0003	3.9	2.61	0.70

The total average percentage of reduction in the number of plastics was 14.29% between the pre-laying and incubation (average 83 days based on the dates of deaths of our sample), and 80.67% between incubation and chick-rearing (average 78 days). In the proventriculus, such reduction

was 32.20% between the two first periods and 75% between incubation and chick-rearing. In the gizzard, the plastic reduction was 10.65% between pre-laying and incubation and 81.54% between the last two periods.

Plastic mass followed the same pattern described above for the number of plastics, with significant differences being detected between the nesting periods (Chi.sp = 17.40, df = 2, p-value <0.0001; see Table S3.2b). While the total average plastic mass was similar between pre-laying and incubation (\bar{x} = 0.0089 and 0.0085 g respectively), it was significantly lower for the chick-rearing period (\bar{x} = 0.0006 g). Significant differences were also found in the mass of plastic between proventriculus and gizzards (Chi.sp = 21.83, df = 1, p-value <0.0001), with the average values being lower in the proventriculus for the three nesting periods (Table 3.2). On the contrary, plastic sizes were not statistically different between nesting periods (Chi.sq = 2.334, df = 2, p-value = 0.31), the two stomach sections (Chi.sq = 2.492, df = 1, p-value = 0.11), or between the stomach sections over the different periods of the breeding season (see Table S3.3). The overall average plastic size was 3.5 mm, similar to the average size in the proventriculus (\bar{x} = 3.23 mm; ± 1.83 SD; ± 0.33 SE; range: 1–9 mm; n = 30 plastics) and gizzard (\bar{x} = 3.56 mm; ± 1.71 SD; ± 0.14 SE; range: 1–12 mm; n = 141 plastics).

3.3. Polymers and morphologies

From the analysed plastics (n = 110 items or 54% of plastic randomly selected from the total), the most abundant polymer was polyethylene (PE; Fig. S3.4a). This includes both low- and high-density polyethylene identified in 72% of cases (n = 79). PE was also the most abundant polymer in all nesting periods (Fig. 3.3a). Polypropylene (PP; Fig. S3.4b) was the second most abundant polymer (16%; n = 18), followed by nylon (9%; n = 10), further subdivided between nylon12 (n = 4), nylon11 (n = 2), nylon6 (n = 3), and nylon 6/12 (n = 1). The remaining items were made of polyamide (2%; n = 2) and polystyrene (1%; n = 1). Regarding the morphologies of all ingested plastic (n = 203 items), the most abundant were the hard fragments (79%; n = 161 items), even when dividing the data per the three nesting periods (Fig. 3.3b). That plastic morphology was followed in importance by threadlike items (11%; n = 23), sheetlike items (4%; n = 8), plastic pellets (3%; n = 6) and foam plastics (2%; n = 5) (Fig. 3.3b).

4. Discussion

In this study we confirmed that plastic concentrations decrease throughout the breeding season, with breeders experiencing a stomach reset of plastic before starting their winter pelagic phase. When examined in detail, we found significantly lower plastic loads (both in number and mass) during the chick-rearing period compared with the preceding nesting periods. This empirically indicates that adult Cory's shearwaters are decontaminating themselves from plastics when feeding their offspring. Our overall result is in line with previous studies in different Procellariiform species and other orders, in which decreasing plastic trends were detected during

breeding (e.g., Skira, 1986; Van Franeker and Bell, (1988); Ryan, 2015; Nono-Almeida et al., 2023). The growing use of effective bioindicators to monitor marine litter highlights the need for broader knowledge and standardized data collection practices to improve the accuracy of assessments (Savoca et al., 2025). In that sense, our outcomes also support the importance of considering the breeding season when studying plastic ingestion and retention times in seabirds (Ryan, 2015), mainly in species used as indicators of plastic contamination.

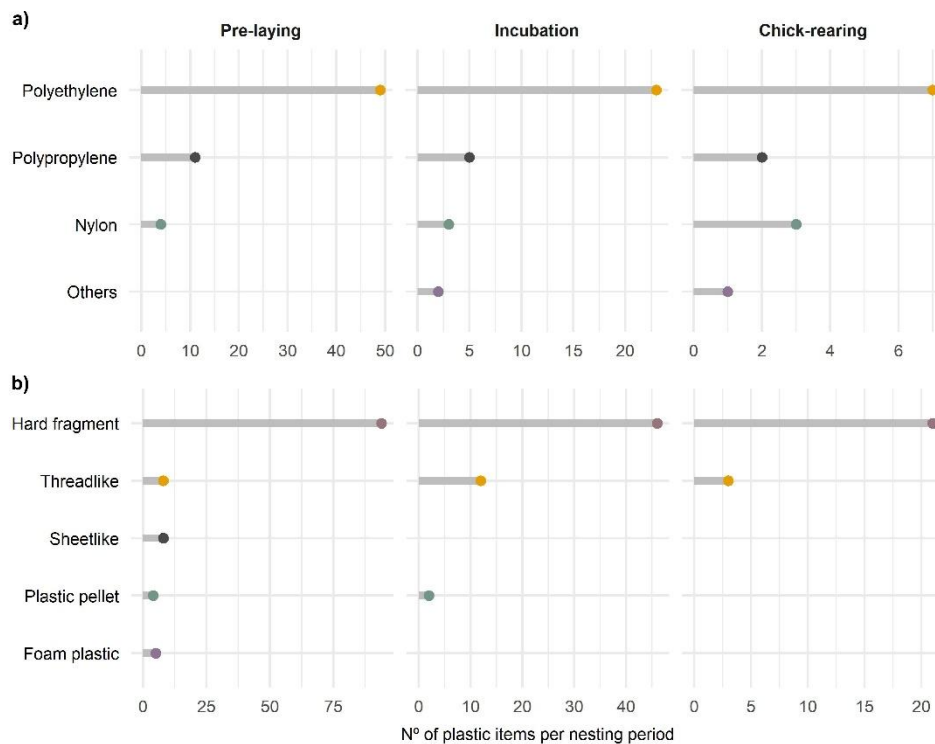


Fig. 3.3. a) Synthetic polymers (n = 110 items) identified with Fourier transform infrared spectroscopy (FTIR) and b) all plastic morphologies (n = 203 items) found ingested by adults Cory's shearwaters over the three periods of the breeding season. The category 'others' in panel a) includes polyamide and polystyrene. Note the differences on the x-axis's scales.

When Procellariiformes feed their offspring, only the proventriculus contents are regurgitated (Furness, 1985). Indeed, we found that around 75% of the plastics in each adult's proventriculus were lost during the chick-rearing period. This cleaning process is advantageous for the progenitors but not for the new generation that receives those plastics. Contrary to the proventriculus, the gizzard acts as a container for the indigestible items such as anthropogenic and organic materials until they can be excreted across the intestines (Furness, 1985; Ryan and Jackson, 1986). We found an 11% reduction in the quantity of plastic accumulated in the gizzard between the pre-laying and incubation periods, which spanned more than two months. However, the transition to the chick-rearing period resulted in a percentage of reduction in the number of plastics in the gizzards seven times greater than the percentage of reduction detected in the previous periods. We suggest that the acute decrease in plastic loads detected in the gizzard during chick-rearing can be explained by the fact that most plastics from the proventriculus might have

been transferred to chicks, and are no longer transitioned to the gizzard. Overall, we found that a negligible amount of plastic in adults was reduced in number between pre-laying and incubation (14% in an average of 83 days). In contrast, between incubation and chick-rearing, such a decrease was much more pronounced over a similar period (81% in an average of 78 days). This indicates that the overall loads found in breeders are highly influenced by the parental transfer of plastics to the offspring. On the contrary, although not statistically significant, the slightly lower plastic concentration detected during incubation concerning pre-laying might be explained by a decrease in the foraging effort due to incubation duties (Navarro et al., 2007). The fasting periods when a progenitor is incubating can lead to a decrease in plastic ingestion and thus, the small differences in some of the plastic metrics obtained between those two initial periods.

Few studies have attempted to calculate the retention times of plastics in seabirds, with the majority using indirect methods and providing a wide range of estimates. For example, soft plastics were estimated to persist for 2–3 months, while hard plastics remained for 10–15 months in short-tailed shearwaters in Alaska, before they returned to their breeding grounds in the southern hemisphere (Day, 1980). That study based its conclusion on observations of birds of unknown ages and relied on visual examination of the surface characteristics and angularity of each ingested plastic to quantify wear rates (Day, 1980). In a subsequent assessment using the same approach, Day et al. (1985) adjusted their estimates to 6 months of the retention time for individual plastics. These authors indicated that sufficient wear and fragmentation allowed excretion through the intestines in short-tailed shearwaters and tufted puffins within this timeframe. Still, the authors warned about variations in the estimated retention rate depending on the number, size, type of particles, and other hard objects that may be present in the stomachs. A few years later, a study compared plastics ingested by different petrel species according to whether they were year-round equatorial residents or migrants, concluding that a gross value as the retention time of plastic over 6–12 months would fit the patterns of an annual migration (Ainley et al., 1990). Other studies collected empirical data based on invasive and non-ethical methods in which captured birds were fed with a specific plastic type before being sacrificed. One example was a study involving white-chinned petrel fledglings (*Procellaria aequinoctialis*), which showed a retention period of two years based on mass loss of polyethylene plastic pellets throughout a feeding experiment (Ryan and Jackson, 1987). However, such a study did not replicate the true composition of plastic being ingested by wild birds, nor environmental factors influencing foraging in open ocean areas.

More recently, Van Franeker and Law (2015) suggested that 75% of hard plastics might be excreted within one month from the stomachs of fulmarine petrels. Such an estimate was based on data from Van Franeker and Bell, (1988) and an 80–90% plastic decrease in Antarctic Cape Petrels within ~1.5 months from pre-breeding and breeding. However, such retention time be

conservative due to small samples (i.e., one pre-breeding bird; Van Franeker and Bell, (1988); Van Franeker and Law, 2015), or because the authors did not consider the intergenerational transfer of plastics as an explanation in some of the species (see Ryan, 2015). Additionally, Great shearwaters from different locations were compared to obtain information regarding ingested plastics over their entire biological annual cycle, with the authors proposing 4–6 months as the turnover rate of plastic fragments retained in the gizzard (Robuck et al., 2022). Yet, data from different individuals, years, and collection methods were integrated to make the extrapolations. Overall, the true extent of plastic retention times in seabirds is often misrepresented because most studies rely on data that is not comparable, fail to account for important ecological characteristics of the species, use different sampling and statistical approaches, and have limitations in their calculations. Additionally, the type of plastic ingested in terms of morphology, size, or polymer type can also influence the comparisons (Ryan, 2015). For this reason, the retention times of plastics provided so far for seabirds need to be taken with caution. Considering the explanations outlined above, recognizing the limitations of our data, and assuming the importance of chick-rearing in the plastic loads found in adults, we find it prudent to avoid providing an estimated time for this process in the studied species.

The presence of indigestible prey items in birds' stomachs can also influence plastic wear rates (Day et al., 1985). Thus, analyses of the stomach contents might help to elucidate the lifespan of plastic items in the gizzard of Procellariiformes. In this study, unlike the plastics, we did not observe a clear decrease in the number of beaks throughout the breeding season. Similarly, no correlation was found in northern fulmars between the number and the plastic mass with the number or size of cephalopod prey (Donnelly-Greenan et al., 2014). Yet another study found a simultaneous decrease in the number of squid beaks and plastic items in the stomachs of the same species (Van Franeker and Law, 2015). These contrasting results indicate that more detailed studies are needed. Further investigations should focus on the degradation process of the materials that compose the plastics (polymers) and the beaks (chitin) in the gizzards, which might clarify if there are different retention times among them.

The excretion size, dictated by the morphology of the gastrointestinal tract, might also play a crucial role in wear rates (Ryan, 2015). However, limited research has investigated the sizes at which plastics are excreted into the seabird intestines. The available information comes only from northern fulmars, with studies initially detecting a maximum plastic excretion size of 2 mm (Bravo-Rebolledo, 2011) and recently particles up to 5.4 mm in the intestines (Terepocki et al., 2017) or 5.6 mm in the transition between the gizzard and the intestine (Tulatz et al., 2023). In our study, the average plastic size was 3.5 mm, and no statistical differences were found in the sizes of plastics between stomach sections and over the breeding season. Similarly, Mallory (2008) found that the length, width, and volume of plastic fragments did not differ during breeding

in northern fulmars, and although the mean plastic surface area was statistically lower later in the season, the mean averages were very similar (0.66 ± 0.07 vs 0.62 ± 0.07 cm²). The excretion size threshold for plastics in Procellariiformes is a relevant issue, as it defines that a portion of plastics within the microplastic range (≤ 5 mm) might undergo elimination following a short residence period in the gizzard. In that sense, plastic degradation in the gizzard might not occur to a visible significant level, at least for the types of plastic commonly ingested by Cory's shearwaters in the Azores (particularly small pieces ranging from 1 to 5 mm and a morphology highly resistant, the hard plastic fragments). In this case, the degradation may primarily occur through surface friction leading to the formation of very small microplastics (<1 mm).

Future studies examining the non-visible fraction of plastics (<1 mm) in the gizzard, as well as characterizing plastic items in the intestine, could provide valuable insights into the size of plastics when excreted. Indeed, studies of seabird fecal samples have already identified plastic sizes and morphologies that differ from those found in stomach contents, suggesting that guano may reflect plastic that was reduced in size (Provencher et al., 2018). To date, no adverse effects (i.e., obstructions, ulcers, malnutrition) have been detected in the studied species, either in adults (this study) or juveniles (Rodríguez et al., 2012a, 2024), as a result of plastic ingestion. This may be due to the relatively low concentrations and small sizes of the plastics ingested. Yet the presence of plastics in the gut of Cory's shearwaters influences the microbiome, for example, by increasing the number of pathogen microbes (Fackelmann et al., 2023). Despite the inherent challenges, studies investigating plastic excretion sizes in seabirds are needed to enhance our knowledge and related health implications (Ryan, 2015; Terepocki et al., 2017).

Monitoring adult breeders throughout their migration is also important to understand the dynamics of ingested plastics. Specifically, Cory's shearwater winter foraging grounds range from the NW and NE Atlantic to various areas of the South Atlantic and Indian Ocean such as the Brazilian current, the Benguela, and the Agulhas Currents (Dias et al., 2019). In a study based on data collected in the NW Atlantic (Nova Scotia, Canada), Bond et al. (2014) did not find plastics in the stomachs of Cory's shearwaters. Yet the sample size was small ($n = 3$ birds). In the SW Atlantic, Colabuono et al. (2009) reported a high incidence of plastic ingestion in Brazil (100% FO; $n = 5$ birds) with an average of around six items per bird. In the same region, Petry et al. (2009) also found a high incidence of plastic ingestion (81% FO; $n = 185$ birds) with an average of eight user plastics ingested per individual. However, Baes et al. (2024) reported a lower frequency of plastic ingestion for Cory's shearwaters also found in Brazil (28% FO; $n = 119$ birds). Recently, Gallo et al. (2024) obtained an intermediate frequency of plastic ingestion (46% FO; $n = 13$) and an average of 1.5 plastic in a sample that contained different age classes of Cory's shearwaters collected between Argentina and Brazil. These contrasting results for Cory's shearwaters collected in different areas might be explained due to not reported information by

some of those studies (e.g., birds' ages, breeding origin, or specific collection date) impeding a better comprehension of plastic concentrations over an annual biological cycle for this species.

In Rodríguez et al. (2024), adult Cory's shearwaters sampled in breeding colonies at the NE Atlantic were reported with a 59% FO and an average of two plastics per bird independently of the date when the adults were collected. We have shown herein that when sampling adult Cory's shearwaters at the onset of breeding in the Azores, the frequency of plastic ingestion is 68%–75% (3.5–3 items and 0.009 g) in pre-laying and incubation respectively, whereas after chick-rearing it decreases to 36% (0.57 items and 0.0006 g). This contrasts with the information collected from fledgling Cory's shearwaters in the same region, which have a much higher %FO and load of plastics (93% FO, 11 items, and 0.0193 g) when leaving the nest (Rodríguez et al., 2024). Accordingly, the differences in plastic loads across the year and among life stages highlight that at least the age and date when the birds are found dead need to be reported in future studies. Ideally, as Cory's shearwaters nesting in the Canary Islands have higher plastic concentrations and ingest plastics of different morphologies than those nesting in the Azores (Rodríguez et al., 2024), the breeding area should also be identified (if possible) to avoid confounding factors. Moreover, it has been demonstrated that the analyses of perishing low-quality birds may overestimate plastic ingestion by the entire population (Rodríguez et al., 2018). Therefore, the collection method should also be considered and registered for future comparisons over the entire annual cycle of a species.

5. Conclusion

This study highlights that the loads of plastics in breeder seabirds are a highly dynamic process largely influenced by the intergenerational transfer of plastics from the proventriculus, and secondarily by the excretion of accumulated items from the gizzard. The marked reduction in plastic loads by the end of the breeding season suggests that future studies should account for this, as including breeders during or after chick-rearing may underestimate adult plastic ingestion and obscure temporal patterns. Overall, our findings extend beyond the presence of plastic in digestive systems, providing valuable insights into Cory's shearwaters as indicators of plastic litter in the North Atlantic (Rodríguez et al., 2024). We elucidate why breeders should not be used as bioindicators to monitor plastic contamination during nesting. In the first phase of the breeding cycle, after their migration, adults might contain concentrations and morphologies of plastics coming from their distant wintering areas. During the chick-rearing period, progenitors reset their plastic loads leading to biased information of plastic at sea. Addressing knowledge gaps, such as how much time plastics are retained inside marine organisms, becomes imperative for developing effective mitigation strategies. The urgency of this endeavour is underscored by the growing threat that plastic contamination poses to marine ecosystems (MacLeod et al., 2021), as well as the pivotal role seabirds play in assessing the health of our ocean.

CHAPTER IV

Cetaceans playing with single-use plastics (SUPs): A widespread interaction with likely severe impacts³

Abstract

Play is a common behaviour in wild cetaceans that includes the manipulation of natural, as well as artificial objects such as marine debris. Yet, very little is known about these interactions despite the potential impacts on cetacean health. We combined a detailed review of the scientific literature and social media with 12 years of observations to examine cetacean interactions with plastic litter. A total of 11 odontocete species (*Tursiops truncatus*, *Stenella longirostris*, *Delphinus delphis*, *Grampus griseus*, *Steno bredanensis*, *Stenella frontalis*, *Sotalia guianensis*, *Pseudorca crassidens*, *Orcinus orca*, *Globicephala melas* and *Physeter macrocephalus*) were documented in 59 events carrying or throwing plastic litter with their head and/or flippers suggesting a form of play. Interactions occurred in the Atlantic, Pacific, Indian Ocean, Mediterranean, and Red Sea, with single-use plastics composing the main typology registered. While these interactions appeared harmless to the observers, they can pose a significant risk through subsequent entanglement or ingestion.

³Rodríguez, Y., Silva, M.A., Pham, C.K. and Duncan, E.M., 2023. Cetaceans playing with single-use plastics (SUPs): A widespread interaction with likely severe impacts. *Marine Pollution Bulletin*, 194, p.115428. <https://doi.org/10.1016/j.marpolbul.2023.115428>

1. Introduction

Animal play can be described today as a ‘repeated, seemingly non-functional behavior differing from more adaptive versions structurally, contextually or developmentally, and initiated when the animal is in a relaxed, unstimulating or low-stress setting’ (Burghardt, 2010). Although this behaviour may be easily recognized when it happens, it is still poorly reported and lacks an interdisciplinary research approach (Burghardt, 2014).

Play has been described as important to life as other vital functions since it allows for the development of advanced cognitive functions normally linked to the animal's well-being (Oliveira et al., 2010), and involves a whole suite of behaviours traditionally divided between social (i.e., when two or more animals interact), locomotor (i.e., when in solitary entailing running or jumping), or object manipulation (i.e., activities with an inanimate object by solitary animals or groups) (Beach, 1945; Burghardt, 2005). Although object play involves interactions with natural elements without an apparent final function (e.g., Koops et al., 2015), this behaviour trains the motor area promoting the improvement of physical and cognitive abilities (Bekoff, 1984). Object play has already been detected in many different species of wild animals, but more specifically in mammals such as primates (e.g., Jolly, 1985; Nishida and Wallauer, 2003; Koops et al., 2015) and elephants (e.g., Lee and Moss, 1999; Evans and Harris, 2008; Lee and Moss, 2014). Despite its relevance, most studies have been carried out in terrestrial wildlife because the logistics of studying animals in the marine environment greatly hinder the observation and understanding of many relevant interactions (King and Jensen, 2022).

Current anthropogenic threats affecting marine environments drive multiple disruptions of ecological processes and have been shown to change marine animal behaviours. For example, tourist activities that involve food provisioning can affect female reproductive success and calf survival in bottlenose dolphins (*Tursiops aduncus*) (Senigaglia et al., 2019), or modify the traditional migration routes of whale sharks (*Rhincodon typus*) by increasing the residence of animals in places with human presence (Araujo et al., 2014). Fishing activities in certain areas can alter the normal behaviour of bottlenose dolphins (*Tursiops truncatus*) causing unnatural attraction to boats and leading to a growing number of strikes and entanglements (Donaldson et al., 2010). Artificial lights disturb the duration of nesting processes and its orientation toward the sea of female loggerhead turtles (*Caretta caretta*) (Silva et al., 2017), and influence the visually guided behaviour of Cory's shearwaters (*Calonectris borealis*) chicks (Atchoi et al., 2023). Modifications in the distribution of Southern right whales (*Eubalaena australis*) on nursery grounds might be caused by boat pressures (Rowntree et al., 2020), whereas whale-watching boats can increase energetic costs in resting and diving sperm whales (*Physeter macrocephalus*) (Oliveira et al., 2022). Moreover, the increased human-related underwater noise is known to alter

the social behaviours of a wide number of marine organisms including fish, mammals, mollusks, arthropods, and reptiles (Peng et al., 2015).

The ever-expanding human-induced pressure of plastic litter has become a major and pervasive threat to the marine environment worldwide (Villarrubia-Gómez et al., 2018), leading to biodiversity losses (Roman et al., 2019b; Senko et al., 2020). The presence of plastic has the potential to affect marine animals when feeding, as they may ingest these items because they confuse it with their natural prey (e.g., Duncan et al., 2019), use it to build nests (e.g., Jagiello et al., 2019), or become entangled (e.g., Rodríguez Y. et al., 2022). Marine litter also disturbs natural biological traits when animals use anthropogenic objects as a refuge, a shelter, or a substrate, and when they cover and/or smother, or use them as a mobile home instead of using natural materials (de Carvalho-Souza et al., 2018).

Marine megafauna such as cetaceans have been exposed to many human pressures, prompting population declines or the extinction of several species over the past centuries (Pimiento et al., 2020). Among the different threats, cetaceans are also affected by plastic pollution and have been proposed as global indicators of marine litter impacts (Fossi et al., 2020). Reportedly, at least 69% of the species are ingesting plastic or becoming entangled in anthropogenic items (Kühn and van Franeker, 2020). However, there is still little information on the mechanisms that leads to both interactions (Simmonds, 2012). Among the hypotheses, it has been proposed that playful behaviours, typical of marine mammals such as cetaceans, might influence subsequent entanglements or the ingestion of plastic litter (Laist, 1987).

In this study, we aim to add novel information about interactions between cetaceans and marine debris by documenting an underreported behaviour in wild cetaceans – play with plastic litter. Due to the difficulty in studying this topic, a mixed methods approach was adopted integrating a comprehensive analysis of peer-reviewed literature and social media, complemented by insights from a detailed case study. We investigate worldwide opportunistic observations of various cetacean species interacting actively with marine litter to better understand if such behaviours can be a mechanism leading to entanglement, or the ingestion of anthropogenic items within this group.

2. Material and methods

2.1. Literature and social media review

Reviews of the peer-reviewed literature (Web of Science and Google Scholar) and two social media platforms (Twitter and Facebook) were performed aiming to document potential cases of cetaceans playing with plastics around the world. The search words were “plastic”, “marine litter”, “marine debris”, “user-plastics” and “single-use plastics”, paired with “marine animals”, “cetaceans”, “dolphins”, and “sperm whales”. These terms were all combined with “play”, “interact”, “playing”, or “interacting”. Lately, it has been recommended that during these reviews

search words should be applied in different languages for better coverage (Lyons et al., 2020; Peter et al., 2022). Therefore, all of them were translated into two other languages (Spanish and Portuguese), although more would have given a much fuller view of the problem.

General data collected from each event included the species, the region, and the ocean. Furthermore, specific information was gathered including details such as 1) the overall plastic typology classified between user plastics and fishing-related items, 2) an additional categorisation of these general typologies following the guidelines established by the Marine Strategy Framework Directive (MSFD) into a specific object (i.e., plastic bags, packaging items, balloons, plastic rings, and fishing gears) or remains of objects (i.e., sheetlike items, threadlike items, and hard plastic fragments) (Hanke et al., 2013), 3) the main colour of each item and 4) the body area used by the animals during the plays classified as the head (including items held in the mouth), and the pedunculus distinguishing between the dorsal fin, pectoral fins, and the fluke.

In addition, some precautions were taken to overcome potential problems derived from the use of social media publications. For example, we consider the behaviour as ‘play with uncertainty’ when only a picture was available, even though the publication mentioned that the animals were playing with the plastic items; thus, we safeguard from errors of untrained observers since we did not have direct contact with them to confirm and clarify doubts about the perceived behaviour. On the other hand, behaviours were assumed as animal play when a sequence of pictures, an accurate description of the event, or a video was accessible and analysed by the author team. Additionally, it was taken into consideration that posts could have been done years after the event was originally recorded, and that accounts can be deleted in the future which causes the data to be lost (Peter et al., 2022). Therefore, the variable year was recorded as the ‘year of publication’ for social media data, and all the posts included in the database were saved by using print screens in a private catalog as a backup. Due to all the inherent uncertainties around some of the data (e.g., more events recorded in the Atlantic influenced by the case study, or by the social media search engines, languages applied, etc., or certain species being documented more due to most frequently being sighted during boat trips, or a higher abundance/presence in certain places; Fig. 4.1a–b), we decided not to perform any advanced statistical analysis of abundance or trends over time, species, and locations to avoid bias.

2.2. Case study

The data was collected over 12 years by a whale-watching operator working on São Miguel Island in the Azores Archipelago (38°N, 28°W), a remote group of nine oceanic islands located in the NE Atlantic. Specifically, the Azores are known to be an important habitat for a high diversity of cetaceans with a mix of year-round residents and migratory species (Silva et al., 2014). Although the data was collected usually by biologists without proper training in the observation of cetacean behaviour, reports of animals repeatedly approaching and interacting purposely with plastic litter

were seen as reliable signs of cetacean playing. In those cases, the species, date, and a short description of the event were recorded, along with photographs. A detailed plastic classification followed the steps explained in the section above. Furthering this, potential impacts on the animals were defined between 1) superficial scars or deep wounds (injuries directly caused by the items), and 2) entanglements (animals found entwined or trapped in plastics from the same litter typologies that were registered during the playing behaviours). The health of the animals was evaluated only with a visual assessment for injury since the complete status was not possible. The R statistical software was used to create the graphs (R Core Team, 2021).

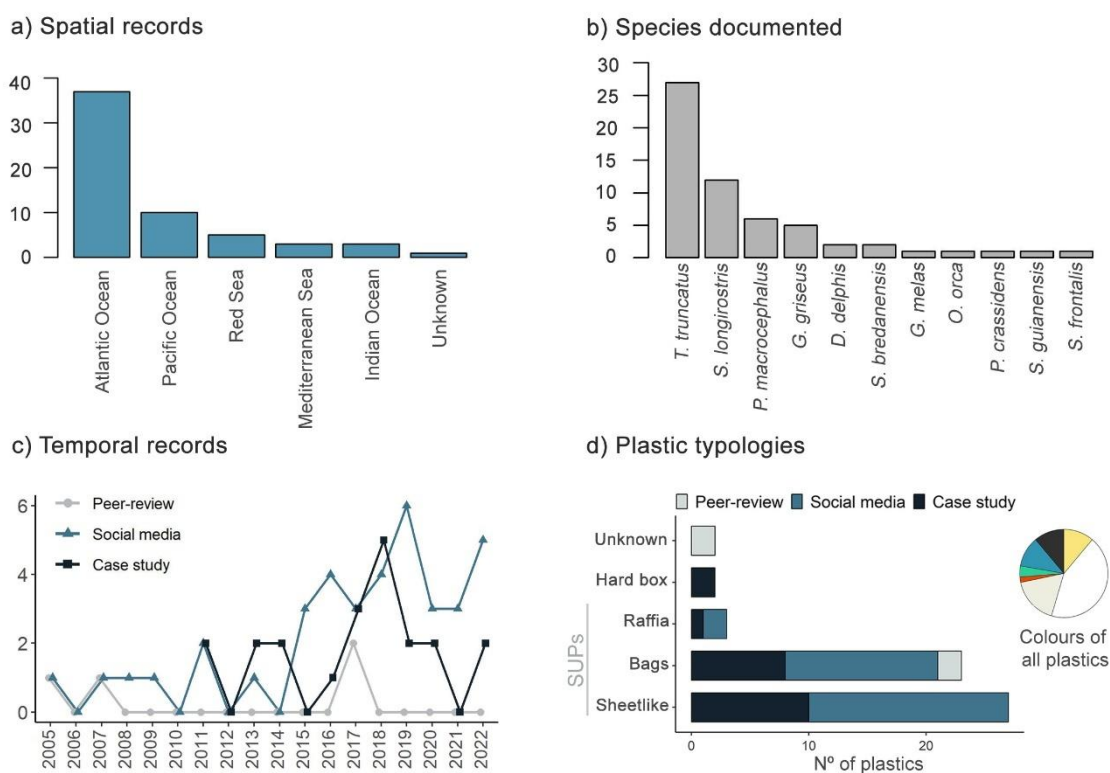


Fig. 4.1. a) Number of records per ocean basin where cetaceans have been reported to interact with plastic including all the events registered in social media, peer-reviewed literature, and the case study; b) species of cetaceans documented to interact with plastic litter; c) the number of records found over the years on the social media and scientific literature (the x-axis represent the year of the publication), and the number of events recorded on the case study (the x-axis represent the year of the event) and; d) characterization of the plastics typologies by shape and colours.

3. Results

3.1. Literature review and social media records

Cetacean play with plastic litter has been rarely reported in the peer-review literature (Fig. 4.1c), with only four events in three different studies. These concerned rough-toothed dolphins (*Steno bredanensis*) in the NW Atlantic playing with a ‘piece of plastic’, and long-finned pilot whales (*Globicephala melas*) and Rissos's dolphins (*Grampus griseus*) playing with plastic bags in the Mediterranean Sea (Fig. 4.1d). Unfortunately, no other detailed information on the interactions or the litter items was provided since these events were only anecdotal.

In addition, 34 events were found on social media websites documenting eight different species of odontocetes playing (53%) or potentially playing (47%) with plastic litter (Supplementary Table S6.1). Overall, the majority were bottlenose dolphins (*Tursiops truncatus*; 41%), followed by the spinner dolphins (*Stenella longirostris*; 35%) and sperm whales (*Physeter macrocephalus*; 9%). The other species were the Guiana dolphin (*Sotalia guianensis*), the false killer whale (*Pseudorca crassidens*), the killer whale (*Orcinus orca*), the Atlantic spotted dolphin (*Stenella frontalis*), and the common dolphin (*Delphinus delphis*) with one event each. Interactions between cetaceans and plastic litter have been published on social media websites for at least over 18 years (Fig. 4.1c), and from a variety of locations around the world. In the NE Atlantic, records were found from oceanic archipelagos such as the Canary Islands (15%), Madeira (3%), and the Azores (9%). In the NW Atlantic, animals interacting with plastic were detected in the Bahamas (6%) and the Grand Turk islands from the Caribbean Sea (3%). In the South Atlantic, records were found in Brazil (6%). Most of the occurrences in the Pacific basin were from Hawaii (21%), with an event detected in the NE Pacific and another in the Bonin Islands in Japan (3% each). The remaining locations included Mauritius in the Indian Ocean, Gibraltar in the Mediterranean Sea (3% each), and the Red Sea with events reported from the Egyptian waters (13%). On the remaining events, the locations were unknown (12%). Single-use plastics (hereafter SUPs) were the only typology featured across these specific interactions found between cetaceans and plastic in the social media records (Fig. 4.1d). These items were further divided between sheetlike items (56%), plastic bags (38%), and leftovers of raffia sacks (6%) (Fig. 4.2). Most of the items were white (47%), followed by transparent (26%), blue and black (9% each), green (6%) and yellow (3%). Overall, the majority of records were pictures (62%), with a few video recordings (38%).

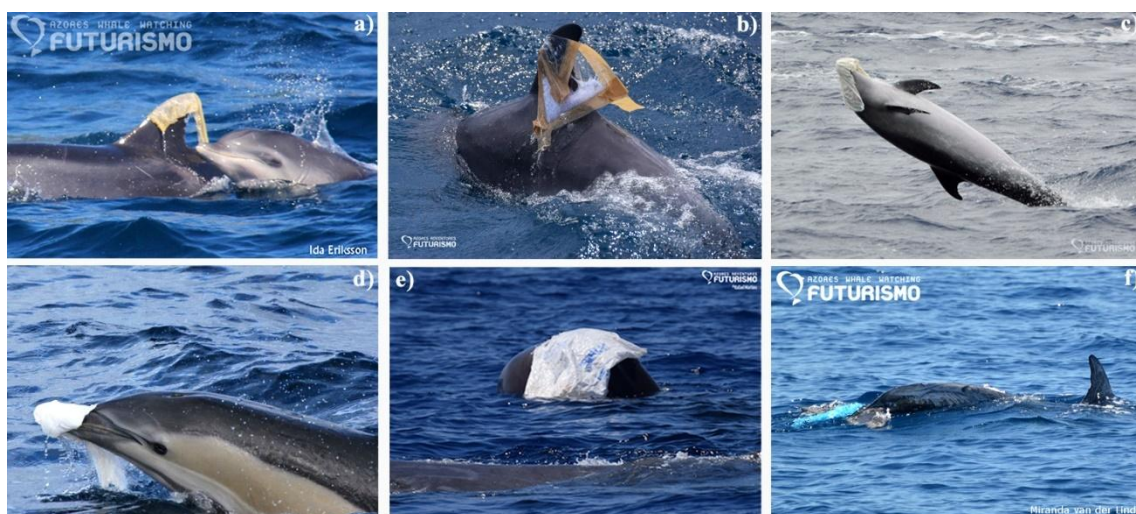


Fig. 4.2. Examples of bottlenose dolphins (a-c), common dolphins (d), sperm whales (e), and Risso's dolphins (f) interacting with SUPs such as plastic bags or sheetlike items in the NE Atlantic. Photo credit: Futurismo – Azores Whale Watching.

3.2. Azores case study - São Miguel Island

Between 2011 and 2022, a total of 21 different individuals were observed actively interacting with plastic litter in presumably play behaviour (Fig. 4.1c; Supplementary Table S4.2). The most frequent species was the bottlenose dolphin (62%), followed by the Risso's dolphin (19%), the sperm whale (14%) and the common dolphin (5%). Only animals from the odontoceti suborder and two families (Delphinidae and Physeteridae) were documented exhibiting this behaviour (Fig. 4.2). SUPs were the main typology observed (90%), followed by two events involving hard plastic boxes (10%) (Fig. 4.1d). From the SUPs, the specific shapes were remains of sheetlike items (53%) where one had a brown plastic tape around (Fig. 6.2b), plastic bags (42%) and raffia sacks (5%) (Fig. 6.2). The observed items were white (33%), yellow (24%), black (14%), blue (14%), transparent (10%) and red (5%).

None of the animals showed obvious signs of injuries caused by the items they were interacting with. However, two animals were observed entangled in SUPs during the study period. The first case involved two different animals from the same group in distinct circumstances at the same time: 1) an animal that was interacting with a large black sheetlike item with playful behaviour (Fig. 4.3a), and 2) another animal with an entangled black sheetlike plastic already constricting its head (Fig. 4.3b). Similarly, a different bottlenose dolphin was sighted on another occasion but with a white single-use plastic also entangled around the neck.

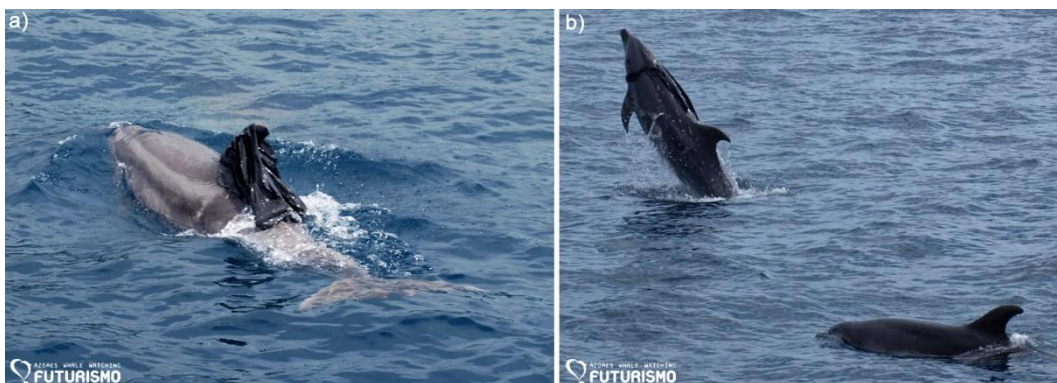


Fig. 4.3. Bottlenose dolphins from the same group show two different scenarios in the same sighting; a) an individual interacting with a black plastic bag and b) another individual observed with a different black plastic bag already entangled around the head. Photo credit: Futurismo – Azores Whale Watching.

3.3. General behavioural observations

The animals were seen interacting mainly with the plastic items using the head, including the melon and beak (34%), the dorsal fins (32%), the pectoral fins (20%), and the fluke (14%). In some cases, the animals moved the plastics between different parts of the body in the same event. Overall, the animals were observed interacting actively with plastic litter, catching the items purposely and then carrying them, sometimes going back for the item after it slipped from the body. On other occasions, the animals were seen throwing the items out of the water. Moreover, animals from the same group were found to interact with different plastic items simultaneously.

In one case, a dolphin interacting with the plastic was accompanied closely by a calve (Fig. 4.2a). On another occasion, a killer whale calf was recorded manipulating a plastic bag with its head.

4. Discussion

In this study, we provide broad-scale evidence that cetaceans engage in active interactions with plastic litter, a behaviour most likely representing play. Overall, eleven cetacean species have been identified displaying this behaviour including bottlenose dolphins, spinner dolphins, Risso's dolphins, common dolphins, Atlantic spotted dolphins, rough-toothed dolphins, Guiana dolphins, false killer whales, killer whales, long-finned pilot whales, and sperm whales. Cetaceans were observed intentionally interacting with plastic litter, whether carrying or throwing the items with their melon, beaks, and fins (dorsal, pectorals, and fluke). This behaviour was documented in three main ocean basins (Atlantic, Pacific, and Indian), the Mediterranean, and the Red Sea.

SUPs were the main category of marine litter observed in these interactions, mostly sheetlike pieces or entire plastic bags. SUPs are objects thrown away after being used very briefly, or just once. This makes it easier for them to end up in ocean environments and poses a real risk to marine biodiversity (UNEP, 2018), with many studies showing the ingestion of SUPs in several species of dolphins (Chen et al., 2021). So far, different international policies have been implementing measures to reduce the impacts of certain plastic products. For example, the Single Use Plastics Directive EU/2015/720 seeks to reduce the lightweight plastic bags in the European Union in an attempt to prevent threats of packaging waste on the environment (EU, 2015). Moreover, Goal 14.1 in the 2030 Agenda for Sustainable Development adopted by the United Nations (UN) targets to 'prevent and significantly reduce marine pollution of all kinds, in particular from land-based activities, including marine debris and nutrient pollution' (UN, 2018). The UN Environment Assembly has been striving to establish an effective global plastic treaty aimed at reducing this type of pollution (including SUPs) toward a legally binding agreement (UNEA-5/14, 2022), which is expected to cap plastic production (Bergmann et al., 2022). Even though more than 60 countries around the globe have already introduced bans or regulations to control single-use plastic waste (UNEP, 2018), this source of contamination still represents a high percentage of marine litter in jurisdictions where no strategy exists to reduce SUPs consumption (Schnurr et al., 2018).

Cetacean play with plastic litter has been minimally reported in the scientific literature with only two events documented in rough-toothed dolphins from Honduras (Kuczaj and Highfill, 2005; Ii and Yeater, 2007), and another two events involving Risso's dolphins and long-finned pilot whales in the Mediterranean Sea (Di-Méglio and Campana, 2017). None of these studies focused primarily on this interaction, and as of now, no published research has specifically addressed this issue. The lack of such research is primarily because obtaining reliable data about these events is challenging for the scientific community. Therefore, such interactions are mostly observed and

reported on social media as isolated records by scientists or the public. Misuse of data collected from social media websites can indeed lead to biased studies and erroneous estimates or trends (Peter et al., 2022), but when properly applied, the data that can be extracted from these public platforms may be used for scientific purposes (Parton et al., 2019; Sullivan et al., 2019). Consequently, in this study, we cannot affirm that we found an increased frequency in the occurrence of these interactions because there are more plastics in the ocean, or that animals in the Atlantic or certain cetacean species are more affected by this threat. Indeed, an increase in the number of boat trips, extensive use of cameras/mobile phones, and more awareness about the threats of marine plastics can explain some of the results.

It is important to note that social learning in cetaceans can occur among members of the same generation (e.g., Wild et al., 2020), as well as between mothers and calves (e.g., Rendell and Whitehead, 2001; von Bubnoff, 2005). Thus, playing with plastic litter might also be taught to younger animals, which can lead to serious consequences for the new generations. Remarkably, Kuczaj and Highfill (2005) reported two adult rough-toothed dolphins sharing a piece of plastic in cooperative play, which later involved a juvenile, by leaving the plastic next to each other's beaks. Although this collaborative behaviour was not documented in our case study, a bottlenose dolphin playing with a sheetlike item was sighted accompanied closely by a calf, whereas, in social media, a killer whale calf was documented playing with a plastic bag.

Other types of object manipulation, not involving play behaviours, have been reported in cetaceans. For example, female Indo-Pacific bottlenose dolphins (*Tursiops aduncus*) have been observed carrying sponges apparently as a tool to protect their beak from abrasion when feeding in the benthos in Shark Bay in Western Australia (Smolker et al., 1997). The same population has also been seen manipulating conch shells as another foraging tactic (Allen et al., 2011). Moreover, branches are known to be carried, held in the mouth, struck, or thrown away by adults and sub-adult males of the Amazon River dolphins (*Inia geoffrensis*) as a potential socio-sexual exhibition for females (Martin et al., 2008). These reports of wild dolphins using natural objects for a purpose, however, have been limited and specific to a few populations with most object manipulation recognized today as part of cetaceans' play behaviours (Hill et al., 2017). Despite some uncertainty surrounding the opportunistic data presented here and whether the events truly represented play, such types of play behaviours using the same body areas have been documented in wild cetaceans with remains of kelp, seagrass, or other natural objects (Hill et al., 2017).

Laist (1987) first proposed the possibility of plastic entanglements resulting from play or curiosity behaviours in marine mammals. Although most of the plastics observed in this study were small sheetlike pieces simply placed or draped on specific parts of the body, we consider that playing with plastics of considerable size or with openings (e.g., plastic bags with intact handles) may be key in subsequent entanglements (e.g., head constrictions or wrapping around tails and flippers).

For instance, in the case study conducted in the Azores, it was observed that at least two bottlenose dolphins had big sheetlike items entangled around their heads, while other animals exhibited playful behaviours involving plastics. In the social media review, it was found an event where a spinner dolphin became stressed with a plastic bag that got stuck on the handle after the animal swam directly to it and got it caught on its beak.

In addition, Laist (1987) also identified these playful interactions as a potential cause of plastic ingestion in marine mammals. The unique characteristics of the complex gastrointestinal tract in cetaceans make them especially vulnerable to this threat, as it can lead to physical blockages, disrupting the regular intake of food and creating a false feeling of satiation, which in severe instances may lead to starvation and, eventually death (IWC, 2020). The incidence of plastic ingestion in cetaceans has been increasing, and while the prevailing explanation for most cases is a passive pathway (i.e., an accidental swallow of anthropogenic items), we consider that the role of playful interactions might have been underestimated. In our study, several dolphins were documented playing with SUPs using their beaks, but also sperm whales interacted with their mouths. Moreover, in our case study, we found a few events of sperm whales engaging with hard plastic objects. Interestingly, studies of plastic ingestion in sperm whales have reported high quantities of SUPs (including bags, and sheetlike items), but also hard plastics (i.e., buckets), in their gastrointestinal tracts (e.g., de Stephanis et al., 2013; Unger et al., 2016; Alexiadou et al., 2019). Risso's dolphins have also been found with plastics in the stomach, covering a surface area of around 9m² including black sheetlike plastics (Alexiadou et al., 2019), analogous to the typologies and colours documented in this study. In the Canary Islands, various cetaceans have been detected with macroplastics in the gastrointestinal tract (Puig-Lozano et al., 2018). Several of these species (sperm whales, bottlenose dolphins, common dolphins, rough-toothed dolphins, and Risso's dolphins) overlap with those reported in our study. From the remaining cetaceans that were documented herein to actively interact with plastic litter, the Guiana dolphins have been also reported to ingest macroplastic but, so far, only fishing-related items have been found in their stomachs (Di Benedetto and Ramos, 2014).

Overall, it is critical to start documenting this type of interaction from now on. The adoption of standardized data collection and reporting protocols is crucial, for example, using as a baseline the metrics reported by this study. We suggest using the term “play with plastic litter” in future reports to increase the chances of finding this behaviour using search engines, and including a clear description of the plastic items (i.e., shapes, sizes, colours), individuals (i.e., common and/or scientific name, life stage), and of the interaction and its consequences (i.e., location, date, behavioural context, area of the body used, resulting damages). Our study suggests that video images captured by underwater cameras offer the most valuable and informative data, enabling full observation of the behaviour and facilitating diverse assessments. Thus, we strongly advocate

for the inclusion of video records in the data collection and reporting process to enhance the reliability and accuracy of future analyses.

Whereas play is a natural behaviour that allows further development of socio-cognitive functioning in cetaceans (Hill et al., 2017), playing with marine litter is unnatural and could eventually have severe impacts on the well-being of these animals. Moreover, certain species may be more vulnerable to these impacts due to their playful nature. The importance of cetaceans for ecosystem functioning implies an urgent necessity to address their vulnerabilities to this contaminant to create more suitable conservation measures (Nelms et al., 2021). The inefficiency in managing the end-of-life of plastics results in increasing amounts of plastic waste entering the oceans yearly (Lebreton and Andrady, 2019), which has been predicted to triple in the coming decades (UNEP, 2021). With this in mind, we call for further awareness and specific policies to reduce the quantities of single-use plastics and mitigate the ecological impacts caused by them.

CHAPTER V

An index to differentiate megafauna entangled in operational fishing gears from abandoned, lost, or otherwise discarded fishing gears⁴

Abstract

Classifying entanglements of marine megafauna in certain anthropogenic items is challenging because many are found in fishing-related items, with the cause being either operational fishing gear (OFG) or abandoned, lost, or otherwise discarded fishing gear (ALDFG). The distinction between OFG and ALDFG is essential as each source requires different mitigation and remediation strategies. To reduce the uncertainty in sorting between these two distinct threats, we developed the Entanglement Source Assessment (ENSA) index. This index integrates a series of criteria related to the entangling item and the affected organism. It assesses the likelihood that an entanglement was caused by an OFG or by an ALDFG using a Multi-Criteria Decision Analysis approach. The index was tested on 35 entanglement events involving various taxa, using video footage and photographs recorded in the Azores Archipelago (NE Atlantic). Overall, 57% of the entanglements were classified as likely caused by ALDFG (ENSA scoring >60), indicating they resulted from marine debris. In comparison, 23% were classified as likely from interactions with OFG (ENSA scoring ≤40). The remaining events were ambiguous and could not be confidently assigned to either source. ENSA is a valuable tool that integrates expert knowledge, transparently providing a clear view of the steps and reasoning behind the classification process. It will promote standardization when reporting the source (OFG vs. ALDFG), which is essential for implementing adequate policy measures to address the entanglement of marine megafauna in fishing-related items at national and global scales.

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1. Introduction

The global fishing industry can have detrimental effects on the marine environment, not only through the direct removal of biomass but also via habitat destruction and pollution (e.g., Bush and Mol, 2015; Action, 2020; FAO, 2024). These pressures have been shown to cause significant biodiversity loss, threaten to undermine human food security, and create imbalances in marine ecosystems (e.g., Pauly et al., 2005; Sumaila et al., 2016).

Entanglement of marine fauna in fishing-related items is a major issue caused by the fishing industry, affecting a wide range of marine organisms globally (e.g., Duncan et al., 2017; Parton et al., 2019; Sarrouf Willson et al., 2021; Bertram et al., 2021; Knowlton et al., 2022; Hays et al., 2023; Mghili et al., 2023). This problem contributes significantly to population declines and the annual mortality of many animals (e.g., Laist, 1997; Ryan, 2015; Bergmann et al., 2017; Gilman et al., 2021). These entanglements can be caused by operational fishing gear (defined in this study as OFG) or by abandoned, lost, or otherwise discarded fishing gear (ALDFG). Distinguishing between these two sources is challenging, as the reporting often relies only on expert knowledge rather than a transparent, quantitative, and repeatable method. This distinction is essential as each source requires a tailored strategy to effectively reduce entanglement occurrences (Asmutis-Silvia et al., 2016).

In areas where marine megafauna and fishing gear overlap due to them being key habitats where the animals feed, mate, give birth, nurse their young, or socialize, entanglements in OFG can be prevented through different effective measures (e.g., García et al., 2024). For example, by applying spatiotemporal restrictions such as limiting gear deployment or establishing marine protected areas (e.g., Wu et al., 2022). Technologies such as vessel monitoring systems (VMS) can be used to generate time-series maps of fishing activity that can be related to modelled species distributions and observed entanglements in OFG (Feist et al., 2021). Migratory megafauna can benefit from the so-called ‘blue corridors’, which are protected routes to minimize the threats caused by human activities until achieving a zero-entanglement target by fisheries (Palacios et al., 2023). Other measures include modifications in fishing equipment (e.g., on-demand or ropeless systems) or marking gear with reflective materials or sound-emitting devices that make them more visible to marine megafauna (e.g., O'Neill et al., 2019; Myers et al., 2019; Lima et al., 2023; Fauconnet et al., 2024).

Conversely, ALDFG can be mitigated through prevention, monitoring, removal, behavioural change, innovative technologies and gear improvements (e.g., Chen, 2015; Williams and Rangel-Buitrago, 2019; Bellou et al., 2021). Certain types of gear marking use, for example, satellite-connected electronic devices and machine intelligence that allows tracking and recovery of remote lost fishing gears (e.g., He and Suuronen, 2018). Additional access to oceanographic forecasts, lessening economic pressure, and control of gear costs are the incentives for fishers to

reduce their ALDFG production. In countries with limited access to education and awareness among fishers, with deficient waste management, the availability of specialized training, access to oceanographic forecasts and affordable port reception facilities, can be incentives for them to reduce their ALDFG (e.g., Gallagher et al., 2023; Gilman et al., 2023). Other remediation measures for ALDFG include gear retrieval initiatives (e.g., Large et al., 2009; Gilman et al., 2023). For example, the Korean Government promoted a project for fishers who removed 66,000 tons of litter, including ALDFG in exchange for monetary compensation (Cho, 2009). Similarly, KIMO International launched a practical initiative called 'Fishing for Litter' that seeks to reduce ALDFG in many countries by actively engaging the fishing industry (e.g., Wyles et al., 2019). Recycling projects targeting market demand for end-of-life fishing gear will incentivise fishers to retain and properly dispose of the unwanted gear (Deshpande et al., 2020; Basurko et al., 2023). Misclassifying the source of fishing-related entanglements as either OFG or ALDFG can undermine the efforts to address each threat (Richardson et al., 2019), with potential public and policy misinformation that diverts resources from the most pressing issue. Unlike entanglements in single-use plastics that are directly classified as caused by marine debris, the source of fishing-related entanglements can be erroneously assigned to OFG or ALDFG (Simmonds, 2012). For instance, in some regions, baleen whales frequently become entangled in static fishing gear, often breaking the equipment and dragging it while attempting to free themselves (e.g., Feist et al., 2021). Such cases include humpback whales entangled in Dungeness crab gears (e.g., Lebon and Kelly, 2019), and North Atlantic right whales caught in crab pots, lobster pots, and gillnets (e.g., Johnson et al., 2005; Bisack and Magnusson, 2021). Although such entanglements are interactions with OFG (Asmutis-Silvia et al., 2016), some authors have misclassified them as resulting from ALDFG or ghost gears (Stelfox et al., 2016).

The objective of this study was to develop a measurable and standardised method with transparent criteria to assess the likelihood that an entanglement is either caused by 1) an operational fishing gear (OFG), including accessories (e.g., buoys, ropes) as well as the fishing gear itself (e.g., net), or by 2) an abandoned, lost, or discarded fishing gear (ALDFG), and thus, items which are part of marine debris. The proposed index is intended to be a tool to overcome the uncertainty in the classification. It is designed to support scientists and decision-makers in reporting uniformly the original source of marine megafauna entangled in fishing-related items.

2. Material and methods

Pictures and video footage of entanglements in fishing-related items in the Azores, an archipelago composed of several oceanic islands at the edge of the North Atlantic Subtropical Gyre (NASG), were compiled from a stranding network, researchers, fishery observers, tourist operators and volunteers. A total of 35 events comprised the final dataset on which the developed index was tested. The dataset has information from four different taxa (Fig. 5.1a) including sea turtles

(n = 19), cetaceans (n = 9), sharks (n = 5), and seabirds (n = 2). Five events known to have been caused by OFG were included as a control to test the index performance. These events involved cetaceans (n = 2), turtles (n = 2), and sharks (n = 1) found entangled in fishing gear used within the study region, such as longlines or benthic traps.

2.2. Entanglement Source Assessment (ENSA) index

The Entanglement Source Assessment (ENSA) index was based on the principle that certain key features of both the entangling item and the affected organism can provide clues on whether the entanglement happened in an OFG or an ALDFG (Table 5.1).

2.2.1. Approach followed

A Multi-Criteria Decision Analysis (MCDA) (Dodgson et al., 2009; Dean, 2022) was applied to explore the trade-offs between the different criteria selected to improve the identification of the source causing a fishing-related entanglement. An MCDA approach has been successfully applied previously to address problems related to marine debris (e.g., Bhagat et al., 2016; Marazzi et al., 2020), or to assess the effectiveness of policies in meeting specific objectives such as those from the Marine Strategy Framework Directive (Dias et al., 2022). The MCDA is used in cases where the decisions involve multiple, often conflicting, criteria. It is a transparent and repeatable method that maintains a clear record of the decision-making process. The method is based on the systematical assignment of scores and weights that reflect the importance of each criterion.

2.2.2. Criteria selection

It is vital to highlight that the effectiveness of the index does not rely on any single criterion, but rather on the combined use of all the criteria. The selection of the criteria (Table 5.1; Table S5.1) was partially based on characteristics already identified as important to distinguish both sources in previous studies (e.g., European Commission, 2020; INDICIT Consortium, 2018; Galgani et al., 2024). The integrity of the fishing gear, its composition, origin, condition, and the presence of biofouling were considered the most relevant criteria for classifying the entangling item (see Table S6.1 for detailed descriptions of each criterion). For classifying the entangled animal, its external physical status was the only criterion deemed as significant (see Table S6.1). Several other criteria were tested but ultimately not included in the index because: 1) they could not be easily allocated to one of the threats (e.g., the volume of the entangling item, the percentage of the body covered by the entangling item, the area of the body affected, and the taxonomic group) or, 2) they were underrepresented in many entanglement events (e.g., the number of animals found entangled in the same entangling item).

Table 5.1. Criteria used for distinguishing entanglements in operational fishing gear (OFG) from abandoned, lost, or otherwise discarded fishing gear (ALDFG). Each criterion is scored independently with values ranging from 0 (high likelihood of entanglement in an OFG) to 1 (high likelihood of entanglement in ALDFG). The final scores obtained in all these criteria, in combination with the weight assigned to each of them, will end in an overall metric named as the Entanglement Source Assessment (ENSA) index.

Indicator	Criteria	Definition	Main scoring	Detailed classification	Weight (%)
Entangling item	a) Integrity	Types of fishing gear and their degree of completeness	0	Entire fishing items, and/or the presence of hooks even if the gear is not complete (e.g., an animal with a hook in the mouth but only part of a longline)	25
			0.5	Almost complete fishing items (e.g., crabs or lobster pots without some of the elements such as the buoys)	
			1	Small parts of a complete fishing gear (<15% of the original gear), or single accessories (e.g., ropes, buoys)	
	b) Composition	Conformation of gears and accessories of the entangling item	0	A fishing item directly identified as a single gear type even if it is not complete, or a fishing item consisting of more than one element only if those are related to the original gear (e.g., a rope with a buoy and a pot)	12
			0.5	Two or more fishing items that might, or not might, belong to the same set of gear	
			1	Two or more fishing items not belonging to the same set of gear (unrelated fishing items, or the presence of user plastics) forming a conglomerate	
	c) Origin	A measure that reflects if the entangling item can be from a local source	0	Fishing items frequently used in the region where the event was recorded	20
			0.5	The entanglement involves fishing items that are widely used in the region, but that are also generally common in other areas	
			1	Fishing items that are not used in the region where the event was recorded	
	d) Condition	Physical status of the gear	0	A new-looking fishing item (i.e., filaments have smooth surfaces, bright colours)	20
			0.5	Fishing items at an intermediate deterioration stage, or slightly entangled	
			1	Fishing items that look old/deteriorated (i.e., a substantial portion is broken off, disintegrated, surfaces are no longer smooth due to being weathered at sea), or fishing items that despite looking new are highly entangled	
	e) Biofouling	A measure of the accumulation of organisms on the surface of the gears	0	Fishing items with no visible biofouling, appearing clean from any organic growth	5
			0.5	Fishing items displaying the first signs of biofouling	
			1	Fishing items featuring a layer of organisms covering part of its surface (e.g., the presence of barnacles), or with extreme biofouling	
Entangled animal	f) Status	The level of harm to the entangled animals	0	A dead animal in advanced decomposition status	18
			0.5	Recently deceased animals, in the early stages of decomposition	
			1	Living animals (both healthy or with injuries)	

2.2.3. Scoring and weights

Each criterion included in the index was scored with values ranging from 0 (high likelihood of entanglement in an OFG) to 1 (high likelihood of entanglement in ALDFG) (Table 5.1). Weights were then assigned to the individual scores based on each criterion's relative importance (Table 5.1). The highest weights were placed on criteria whose classification discriminated between one of the threats (the 'condition'), or criteria that are relevant for the ENSA index to function effectively due to their relationship with the fishing activity (the 'integrity' and 'origin'). On the contrary, less weight was given to other criteria whose classification might seem somewhat ambiguous (the 'status' of the animals). Specifically, a lower weight was given to criteria that, although important to classify some events (the 'biofouling' and 'composition'), are not always present (e.g., some fishing gears such as longlines and certain types of nets tend not to accumulate biofouling). Therefore, assigning them a high weight would unfairly penalise events without these criteria.

2.2.4. ENSA index final classification

The final ENSA score for each event is calculated as the weighted sum of all criteria (Eq. (1)). To facilitate the interpretation of the final ENSA index, the ultimate score is allocated into one of the following categories: 1) scores from 0 to 20 classify an event as highly likely caused by OFG (named hereafter as 'HighOFG'), 2) values >20 to 40 would classify an event with a likely probability of being caused by an OFG ('ProbableOFG'), 3) values >40 to 60 classify an event as an intermediate probability between both threats (therefore named as 'Undetermined'), 4) values >60 to 80 classify an event with a likely probability of being caused by entanglement in ALDFG and thus, by marine debris ('ProbableALDFG'), and 5) values >80 to 100 classify an event as highly likely caused by entanglement in ALDFG ('HighALDFG').

$$\text{ENSA INDEX} = \sum_{i=1}^6 C_i \times W_i$$

Eq (1). Where C_i is the score for criterion i (with i ranging from 1 to 6) and W_i is the weight for criterion i .

3. Results and discussion

3.1. ENSA index classification

After applying the ENSA index to our entanglement events, 23% of the animals were classified as likely entangled by an OFG (Table S6.2). Specifically, these events were categorised by the ENSA index as 20% 'HighOFG' and 3% 'ProbableOFG' (Fig. 5.1b). The events known to be caused by OFG and included as controls were correctly classified as 'HighOFG' (Table S6.2), indicating that the index aligns with pre-established expectations. Entanglements considered 'Undetermined' accounted for 20% of the events (Fig. 5.1b; Table S5.2). On the other hand, 57% of the entanglements were classified as likely caused by ALDFG, further categorised by the index

between 40% as ‘ProbableALDFG’ and 17% as ‘HighALDFG’ (Fig. 5.1b; Table S5.2). ALDFG has become an important stressor to marine ecosystems worldwide (Gilman et al., 2023) affecting all habitats from the deep sea (e.g., Pham et al., 2014; Duncan et al., 2023) to the open ocean (e.g., Senko et al., 2020).

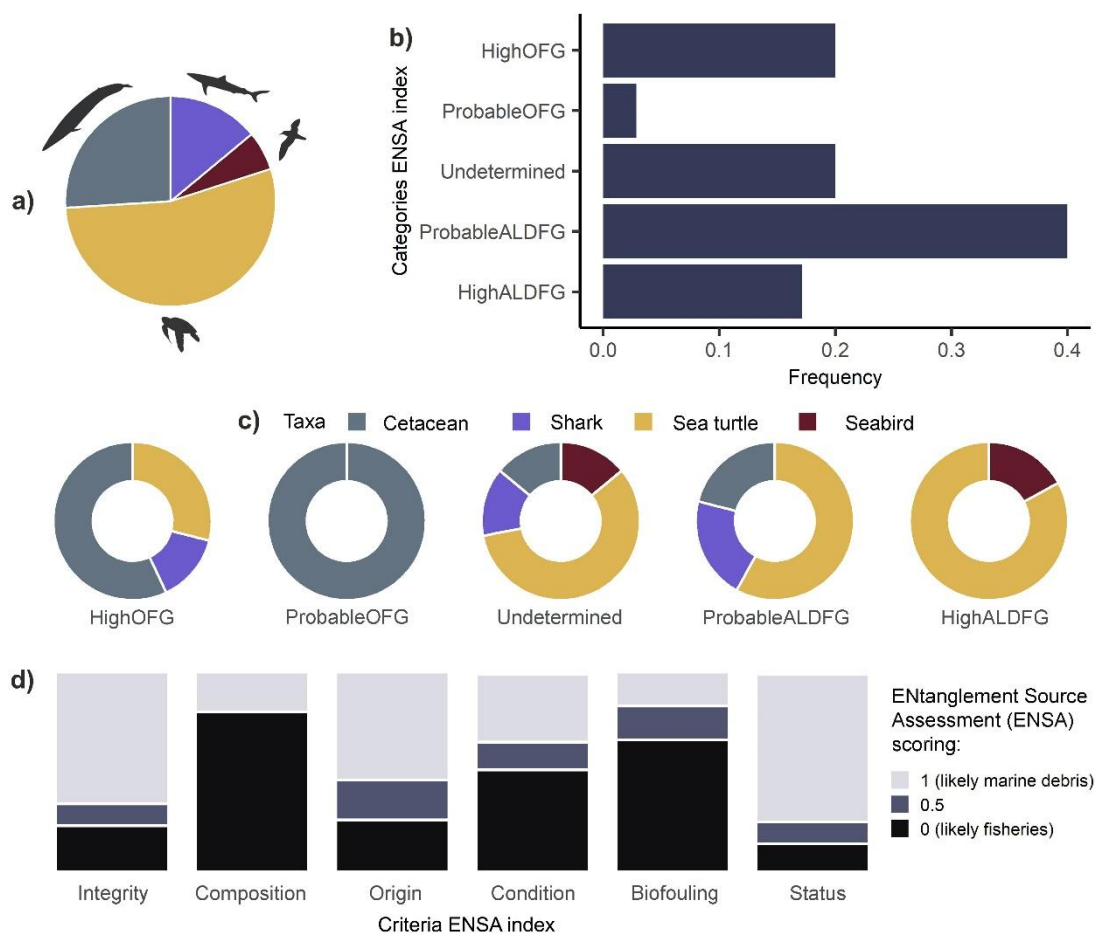


Fig. 5.1. a) Proportion of the different animal groups registered entangled in fishing-related items. b) Result of the ENSA index per category. c) ENSA index classification per category and animal group. d) Frequency of the scores that were assigned to each criterion to classify the entanglement events analysed in this study, ranging from 0 (high likelihood of entanglement in OFG) to 1 (high likelihood of entanglement in ALDFG).

When divided by taxa (Fig. 5.1c), ‘HighOFG’ events involved cetaceans (57%; $n = 4$), sea turtles (29%; $n = 2$), and sharks (14%; $n = 1$). The ‘ProbableOFG’ category had only one case represented by a cetacean. The ‘Undetermined’ events involved all the taxa of which 57% were sea turtles ($n = 4$), whereas cetaceans, sharks, and seabirds were represented by only one animal each. ‘ProbableALDFG’ was divided between sea turtles (57%; $n = 8$), sharks (21%; $n = 3$), and cetaceans (21%; $n = 3$). Events in the ‘HighALDFG’ category mainly involved sea turtles (83%; $n = 5$), with the remaining percentage represented by a seabird (17%; $n = 1$). Sea turtles are considered one of the most vulnerable taxa to entanglements in plastic debris, with five of the seven species being significantly affected globally (Høiberg et al., 2022).

Each criterion was reflected differently in the entanglement events analysed (Fig. 5.1d). The ‘integrity’ criterion was dominated by items that were only a part of the original gear (66%). For the ‘composition’ criterion, no ambiguous cases were found in our dataset, which mostly included fishing gear with its original elements (80%), whereas the rest were conglomerates of various unrelated fishing gears (20%). The ‘origin’ criterion revealed that many entanglements (54%) involved fishing gear not used in the study region. Regarding the ‘condition’ of the gear, most items appeared new (51%), followed by aged or highly entangled and weathered gears (34%). The biofouling criterion indicated that a majority of gears were clean (66%). Lastly, the analysis of the entangled animals showed that most were alive (74%), with 14% found dead in advanced decomposition status.

3.2. Illustration of the ENSA index

Some key examples were selected to illustrate how the ENSA index works (Table S5.2). In the first example (Fig. 5.2a; ID Ent28 in Table S5.2) a leatherback turtle was classified as ‘HighOFG’ (EL score = 0). This event was included as a control to test the index. The turtle was found in an advanced stage of decomposition (‘status’ scoring 0), suggesting it died some time ago. The entangling item was in a well-preserved state (‘condition’ scoring 0) and had no biofouling covering it (‘biofouling’ scoring 0). Despite being composed of multiple components (e.g., trap, rope), all fishing items belonged to the same gear (‘composition’ scoring 0). In this case, the gear belonged to a local fisherman using traps to catch red mullets (*Mullus surmuletus*), a type of gear widely used in the Azores and other regions. The fishing item was complete with twelve traps (‘integrity’ scoring 0) and from a gear used locally (‘origin’ scoring 0). This event is similar to cases involving baleen whales entangled in static fishing gear in other regions (e.g., Johnson et al., 2005; Lebon and Kelly, 2019; Feist et al., 2021; Bisack and Magnusson, 2021).

The second example (Fig. 5.2b; ID Ent33 in Table S5.2) featured a loggerhead turtle classified as ‘Undetermined’ (ENSA score = 55). This turtle was found in an advanced stage of decomposition (‘status’ scoring 0) entangled in gear that is not used locally (‘origin’ scoring 1). Contrary to the first example, the entangling item represented part of the original gear (‘integrity’ scoring 0.5). Although the net did not appear old, it was highly entangled suggesting it had been drifting for some time (‘condition’ scoring 1). Yet, it was not enmeshed with other types of gear (‘composition’ scoring 0), and while not covered with abundant and visible barnacles, it had some biofouling growing on it (‘biofouling’ scoring 0.5).



Fig. 5.2. Examples of the ENSA index performance in sea turtle entanglement events. The cases a) and b) are deceased turtles (*Dermochelys coriacea* and *Caretta caretta*) in advanced stages of decomposition. Case a) entangled in an entire gear used regionally and case b) in a portion of a gear that is not used regionally. ENSA classified these cases as an entanglement in an OFG (a) and as undetermined (b). On the contrary, c) and d) cases show live turtles (*Caretta caretta*) entangled in fishing items not used locally, both classified by the index with a high probability of being entangled by ALDFG. Photo credit: Regional stranding Network of the Azores - RACA.

In the third example (Fig. 5.2c; ID Ent23 in Table S5.2), ENSA classified the event as ‘HighALDFG’ (ENSA score = 88). This turtle was found alive and healthy (‘status’ scoring 1), although highly entangled in a part of fishing net as in the previous case. This indicates that the entanglement occurred recently and in close proximity to where it was recorded. The entangling item was a section of the original fishing net, but this section was much smaller than in the previous example (‘integrity’ scoring 1). This suggests that the entangling device may have resulted from a net being cut due to damage (Metcalf and Bentley, 2020). Despite the net material appearing in good condition, it was severely tangled, suggesting it had been drifting for some time (‘condition’ scoring 1). Since the type of net is not used locally (‘origin’ scoring 1), the likelihood

of finding the animal alive and healthy would be low if the entanglement had resulted from an OFG. In such a case, it would be more likely that the animal would have been found dead or seriously debilitated by the time it reached the Azores. Additionally, the net was covered with relevant biofouling, a clear sign of an item that has drifted passively for a significant period of time as marine debris ('biofouling' scoring 1). Similarly, the loggerhead turtle from the fourth case (Fig. 5.2d; ID Ent22 in Table S5.2) was also classified as 'HighALDFG'. Like the case above, the turtle was alive ('status' scoring 1), but in this case, it was injured. The flipper showed signs of advanced necrosis, indicating that the event had not occurred recently. The entangling item consisted of a conglomerate of more than one type of unrelated gears ('composition' scoring 1), appeared disarray and deteriorated ('condition' scoring 1), and was fully covered by biofouling organisms ('biofouling' scoring 1). Such characteristics indicate that this item was likely drifting as ALDFG when it entangled the turtle. Indeed, floating plastic debris has been considered a substrate in which coastal and pelagic invertebrate species can survive long periods in the high seas (Gil and Pfaller, 2016; Haram et al., 2021). This event was classified as the highest category of the index (ENSA score = 100).

The next example (Fig. 5.3a; ID Ent24 in Table S5.2) features a dolphin entrapped in an entire ('integrity' scoring 0) coastal gillnet used locally ('origin' scoring 0). The net was not found entangled with other types of debris ('composition' scoring 0) and did not appear aged nor was it entangled with itself ('condition' scoring 0). Additionally, the gear was not covered by biofouling ('biofouling' scoring 0). In this case, the entangled animal was found deceased in an advanced stage of decomposition ('status' scoring 0). ENSA classified it as an entanglement resulting from an operational fishing gear (EL score = 0; EL classification = HighOFG). The final example (Fig. 5.3b; ID Ent06 in Table S5.2) was classified as resulting from marine debris (ENSA score = 75.5; ENSA classification = ProbableALDFG). In this case, a baleen whale was entangled in a small portion of a fishing net ('integrity' scoring 1). The net was not entangled with other types of items ('composition' scoring 0), but it had some biofouling present ('biofouling' scoring 0.5), and certain areas were already showing signs of deterioration ('condition' scoring 0.5). The entangling item was not used locally ('origin' scoring 1). Yet, the animal was found alive with the net covering approximately one-third of its body including the mouth area, suggesting the entanglement had occurred recently ('status' scoring 1).

3.3. Requirements for the index application

Although the index was tested using a specific dataset, it encompassed a variety of fishing gears (both with local and distant origin) and taxa. Therefore, the conceptual framework developed herein can be applied to other regions with appropriate expert support. General and local knowledge about fishing gear is essential for the correct application of the index and completing some criteria (namely the integrity, composition, condition, and origin). We recommend

consulting a diverse range of experts, such as scientists, fishers, fisheries enforcement officers, marine conservation officers, rescue center staff, and maritime police. Collaboration among experts, whether from the same or different areas, can help to ensure the index is implemented correctly. Implicit biases of the experts, related to the specific areas of expertise, may inadvertently skew the individual scoring. Additionally, a lack of understanding of the guidelines for scoring each criterion, coupled with an unavoidable degree of subjectivity in their interpretation, can introduce small inconsistencies that can eventually be prevented by collaborative efforts and training workshops. Incomplete information (e.g., low-quality pictures) can also further contribute to variability in scoring outcomes. The collection of visual information (photographic records or video footage) must be rigorous and exhaustive to accurately evaluate all the ENSA criteria.

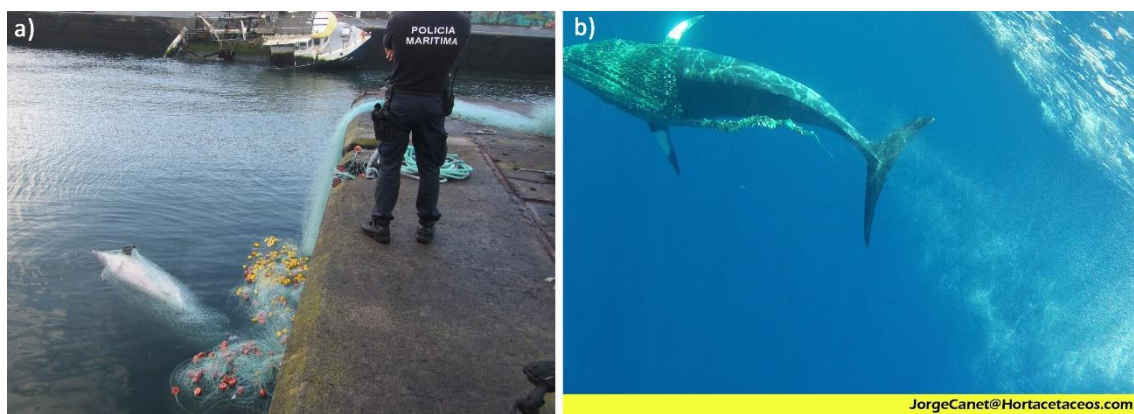


Fig. 5.3. Examples of the ENSA index performance in cetacean entanglement events. a) A deceased dolphin (*Tursiops truncatus*) classified by the index as an entanglement likely caused in its origin by operational fishing gear, thus ‘HighOFG’ (Photo credit: Regional stranding Network of the Azores - RACA). b) An entangled baleen whale (*Balaenoptera acutorostrata*) classified by the index as ‘ProbableALDFG’ (Photo credit: Azores Experiences).

4. Conclusion

In this study, we developed an index that has proven to be reliable in distinguishing the entanglement of megafauna in OFG from that in ALDFG. Reducing the risk of entanglements in both sources is a delicate balance between upholding conservation objectives, and supporting profitable and sustainable fisheries. Achieving this requires accurate source identification for effective management practices, along with active stakeholder engagement (Asmutis-Silvia et al., 2016). This need is particularly relevant when environmental policies such as the Marine Strategy Framework Directive (MSFD, 2008/56/EC), call for studies and monitoring programs focused on identifying indicators of marine litter entanglement and harm (Galgani et al., 2024). The ENSA index presents a transparent framework that integrates expert knowledge, offering a clear view of the steps and reasoning underpinning the classification process. This approach will promote standardised reporting of entanglement events, ensuring accurate attribution of their underlying cause to inform effective management measures to reduce entangled marine megafauna in fishing-related items.

CHAPTER VI

Marine debris entanglements and associated impacts in megafauna across the open NE Atlantic Ocean⁵

Abstract

Entanglement in marine debris is a widespread threat affecting animals worldwide. However, most incidents remain underreported due to the challenges of systematically monitoring their occurrence. This study investigates the entanglement of marine vertebrates in plastic based on 17 years (2008 to 2024) of opportunistic observations from the Azores Archipelago to Portugal's mainland. The data was obtained from: 1) sea users (tourist operators, scientists, fishery observers), and 2) a stranding network. Only events supported by clear visual evidence were included in the analysis. We report a total of 41 entanglements in either, abandoned, lost, or otherwise discarded fishing gears (56%) or in single-use plastics (44%) in ten different vertebrate species (blue shark, swordfish, loggerhead and green sea turtles, Cory's shearwater, bottlenose dolphin, minke, Bryde, and sei whales, and Sowerby's beaked whale). Most individuals (78%) presented visible impacts from the entangling debris, typically external lesions (91%) or mortality (9%). Specific injuries in live animals included constrictions (46%), skin damage such as wounds or lacerations (39%), limb loss (11%), and body deformations (4%). Although opportunistic data may not fully capture the extent and severity of entanglements, we found that floating plastic exerts additional pressure on open ocean ecosystems. This is often overlooked due to a limited network of stakeholders supporting data sharing, which is crucial for understanding this ongoing threat. Overall, this study offers the first baseline evidence on plastic litter entanglements among several marine vertebrate species in the open NE Atlantic, supporting consistent monitoring for the EU Marine Strategy Framework Directive.

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1. Introduction

The pervasive presence of floating marine litter across the world's oceans poses a significant threat of entanglement to marine megafauna. Some of the factors that contribute to the susceptibility of pelagic species to this threat include a broad spatial distribution overlapping with marine debris accumulation areas, specific migratory patterns and certain morphological characteristics or behaviors (e.g., Duncan et al., 2017, Kühn & van Franeker, 2020, Nelms et al., 2023, Rodríguez et al., 2023). Although the entanglement of marine organisms is frequently highlighted in the media, quantitative data and research are limited. This is largely due to operational challenges faced by stranding networks and the difficulty of detecting such events at sea, given the vast areas that require monitoring (Laist, 1997). Moreover, many entangled animals die, either becoming prey or sinking to the seafloor before being detected (Laist, 1997). On other occasions, the data is reduced to a single observation, resulting in most of these events being underreported in the scientific literature (Nelms et al., 2023). These difficulties have caused an extended and general lack of knowledge regarding this pressing issue globally (e.g., Duncan et al., 2017, Claro et al., 2019).

Abandoned, lost, or otherwise discarded fishing gear (ALDFG) is linked to a significant portion of entanglement events involving marine megafauna (e.g., Duncan et al., 2017, Ryan, 2018, Parton et al., 2019). ALDFG, such as fishing lines, buoys, nets, and ropes, constitute marine litter frequently found in remote areas (e.g., Lebreton et al., 2022, Duncan et al., 2023, Royer et al., 2023). ALDFG is also highly abundant on beaches or submerged in coastal waters of the Atlantic (e.g., Link et al., 2019, Wright et al., 2021), accounting for up to 100% of the total debris found on the seafloor in European seas (e.g., Pham et al., 2014). Although fishing gears are responsible for numerous marine vertebrate entanglements, some studies have failed to distinguish whether these occur originally from operational fishing gears (OFG) or ALDFG (Stelfox et al., 2016, Asmutis-Silvia et al., 2017). In addition, plastics not directly related to fishing activities also affect marine species through entanglement. The main items include strapping bands, rings, bags (e.g., Barreto et al., 2019, Jepsen & De Bruyn, 2019), and other forms of single-use plastics commonly referred to as SUPs (e.g., Jepsen & De Bruyn, 2019).

Reports of entanglements caused by marine debris are scarce in comparison with information about plastic ingestion. Yet this issue has already been documented in diverse regions and across a range of marine vertebrate species, including seabirds (Ryan, 2018), elasmobranchs (Parton et al., 2019), sea turtles (Wilcox et al., 2015, Nelms et al., 2016, Duncan et al., 2017) and marine mammals (Jepsen & De Bruyn, 2019, Nelms et al., 2021). Notably, the annual entanglement rate of seals in marine debris has quadrupled over ten years in the Netherlands (Salazar-Casals et al., 2022). In South Korea, a total of 428 entanglements were documented over 20 years in both

coastal areas and underwater environments, affecting at least 77 species of birds, sea turtles, marine mammals, fish, corals, and marine invertebrates (Noh et al., 2025).

The Azores are an isolated archipelago of nine islands in the Northeast (NE) Atlantic. This group of islands provides key habitats for exceptional benthic fauna (e.g., Morato et al., 2021) and marine megafauna (Afonso et al., 2020). The broader Azores region lies at a migratory crossroads between the eastern and western Atlantic margins, and productive boreal waters to tropical seas (Afonso et al., 2020). The area is used by numerous vulnerable and endangered marine vertebrate species for feeding, mating, spawning, pupping, or resting (e.g., Monteiro et al., 1996, Frick et al., 2009, Afonso et al., 2014, Neves et al., 2015, Vandeperre et al., 2014a, Das & Afonso, 2017, Silva et al., 2019, Vandeperre et al., 2019, Romagosa et al., 2020, Afonso et al., 2020, Castellano-González et al., 2025). Particularly, marine debris has emerged as a growing threat to biodiversity in the Azores. While plastic ingestion has been reported among various marine vertebrate species (Pham et al., 2017, Pereira et al., 2020, Rodríguez et al., 2022, 2024), entanglement has proven more difficult to evaluate. On the seafloor, several epibenthic species, including cold-water corals and sponges, have frequently been observed entangled in ALDFG (Pham et al., 2013, Rodríguez and Pham 2017, Duncan et al., 2023). By contrast, in the pelagic environment, entanglements in marine debris have only been reported for sea turtles (Barreiros & Raykov, 2014, Rodríguez et al., 2022). This may create a biased perception that some animal groups are disproportionately more affected. However, given the diversity of species in this pelagic ecosystem, including numerous cetaceans, elasmobranchs, and seabirds, it is likely that other marine vertebrates are also vulnerable to debris entanglements in the region highlighting the need to expand the research effort in such environment.

This study combined photographic and video evidence of marine debris entanglements with ALDFGs and SUPs from several stakeholders over 17 years to 1) update the list of pelagic species impacted by this threat in a key open-ocean area of the NE Atlantic, 2) assess the severity of the entanglement by investigating associated injuries and, 3) identify which debris morphologies are causing the entanglements to support policies working to monitor and mitigate this threat.

2. Material and methods

2.1. Study area

The study area encompassed the Azorean Exclusive Economic Zone (EEZ: 33.5 - 43.0° N and 21.0 - 35.5° W) and the waters off mainland Portugal (Fig. 6.1). The Azores' location, approximately 1,815 km from continental Europe and 2,625 km from North America, makes it the most remote group of islands in the North Atlantic (Lima et al., 2018). With a population of around 250,000 inhabitants, the archipelago lacks major industries. Its primary economic activities have traditionally centered on agriculture and artisanal fisheries. Still, a notable rise in

nature-based tourism has been observed in recent years, including a significant expansion of the marine ecotourism sector (Ressurreição et al., 2022).

Despite its remoteness, the archipelago and the surrounding open ocean are heavily impacted by marine debris due to its position at the edge of the North Atlantic Subtropical Gyre. As a result, the region serves as a potential trap and sink for this contaminant (Pham et al., 2020). The open ocean around the Azores is characterized by low productivity typical of oceanic environments (Amorim et al., 2017), in which local upwelling and areas rich in nutrients are located around island slopes and seamounts. These features, alongside the Gulf Stream and its multi-branched systems, including the Azores current (Caldeira & Reis, 2017), support a high diversity of migratory animals, as well as marine debris originating from distant areas (Cardoso & Caldeira, 2021). The region has been considered a high-risk zone in that sense, with most plastics (>99%) predicted to originate from far-reaching sources such as the Caribbean Islands or the United States (Garrard et al., 2024).

2.2. Data collection

The information on entangled animals was gathered from photographs and video footage collected opportunistically from two main sources (Fig. 6.1): 1) the Azores stranding network, and 2) sea users, including opportunistic citizen science reports collected by several private companies (i.e., tourist operators including cetacean watching and diving companies), scientists and fishery observers. Notably, the POPA (Azores Fisheries Observer Program) and the COSTA Project (Consolidating Sea Turtle conservation in the Azores) manage fishery observers who monitor tuna pole-and-line and the pelagic longline fisheries, respectively. These observers collect data in the study region (Fig. 6.1a) extending to areas beyond national jurisdiction (Parra et al., 2023). In contrast, tourist operators and scientists operate exclusively within the EEZ of the Azores archipelago and record information near specific islands (Fig. 6.1b-c). The opportunistic observations documented by scientists were gathered during fieldwork conducted around the islands of Faial and Pico. Similarly, tourist operators based on three islands (São Miguel, Faial, and Pico) collected data during their activity, usually by biologists who supervised the trips. Finally, the regional stranding network, named as RACA, provided information on stranded animals across all nine islands in the archipelago. RACA is a program of the Government of the Azores dedicated to responding to and managing the strandings of marine animals, including cetaceans, sea turtles, and other vertebrate species.

Of the total number of entanglements recorded since 1997 ($n = 120$), only 34% ($n = 41$) from 17 years (2008 to 2024) were included in this study. Since reporting began, 79 entangled marine animals (23 sea turtles, 21 cetaceans, 34 blue sharks, and 1 seabird) were excluded from the present study due to one of the following reasons: a) lack of visual material to confirm the type

of entanglement ($n = 56$), b) the available pictures or videos did not show sufficient information about the entangling item or injuries ($n = 8$), or c) the Entanglement Source Assessment (ENSA) index (Rodríguez et al., 2025; see section 2.2.1) classified some events as either undetermined ($n = 7$) or caused by operational fishing gears ($n = 8$) rather than by ALDFG. The mean (\bar{x}) number of entangled animals per year, reported along with the standard deviation (SD) and standard error (SE), was calculated only from 2012 to 2024. This aimed to prevent further underrepresentation, as only a single isolated entanglement event was included from the early data.

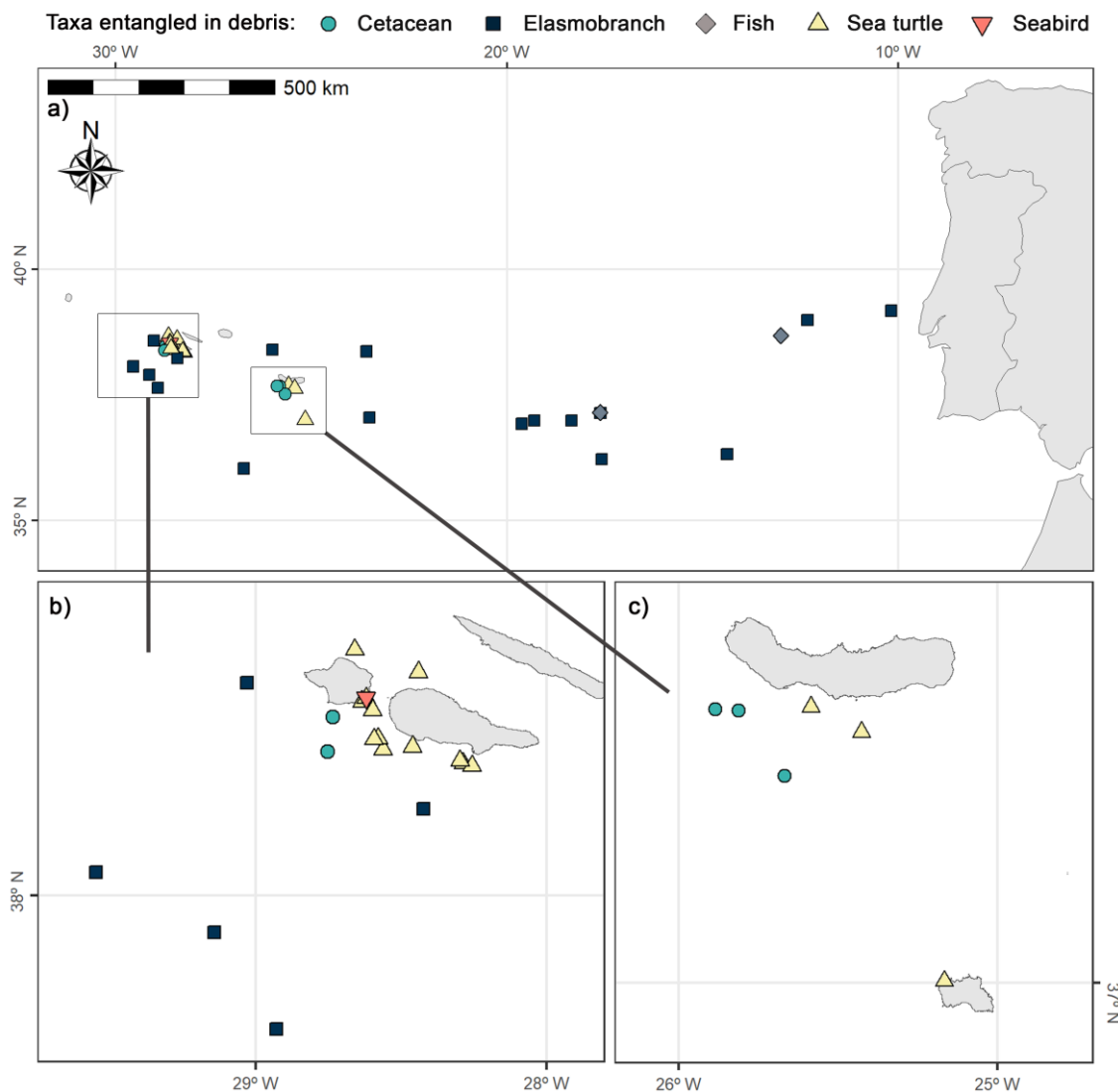


Figure 6.1. Location of the animals found entangled in plastic debris between 2008 and 2024. a) Distribution of all events covering the open ocean between the Azores archipelago (left, with the limits of its Exclusive Economic Zone represented by the dotted lines) and mainland Portugal (right). b) Close-up of the events registered around the islands of Faial (bottom left), Pico (bottom right), and São Jorge (top). c) Close-up of the events registered around São Miguel Island (top) and Santa Maria Island (bottom). Note that some observations overlap.

2.3. Data categorization

The marine debris items responsible for entanglements consisted exclusively of plastics and were, therefore, categorized into two broad groups following Galgani et al., (2023): 1) ALDFG (e.g., ropes, nets, and fishing lines) and 2) other plastics originating from land or maritime activities.

2.3.1. ALDFG

To accurately determine the source of entanglements, the events involving fishing-related items were further investigated by applying the Entanglement Source Assessment (ENSA) index (Rodríguez et al., 2025). Fishing-related items account for a significant portion of entanglements in marine vertebrates, yet these events can occur either when the gear is operational (OFG) or when it is already marine debris (known as ALDFG). To address this uncertainty and avoid misclassifications, the ENSA index (see Rodríguez et al., 2025 for detailed information) was applied to all the entanglements in fishing-related items obtained in our study. Briefly, this index uses a Multi-Criteria Decision Analysis to systematically evaluate entanglement cases by applying pre-defined criteria to distinguish between OFG and ALDFG events. The criteria are defined for the entangling item and the affected organism. Each criterion is then scored on a scale from 0 (high likelihood of entanglement in an OFG) to 1 (high likelihood of entanglement in ALDFG), with weights assigned according to the criterion's relative importance. The final value for each event is calculated as the weighted sum of all criteria. To aid interpretation, the resulting ENSA score (ranging from 0 to 100) is allocated to a categorical classification that provide the likelihood that an entanglement was initially caused by OFG, ALDFG, or whether the origin of the entanglement remains undetermined. In this study, only those events assigned a score above 60 (categorized with a higher probability of being caused by ALDFG) were included in the analyses.

2.3.2. Other plastics

This category includes any land-based or other maritime-sourced plastics, such as single-use plastics (packaging such as bags and plastic sheets, hard plastic rings, strapping bands, six-pack rings, heavy-duty sacks, and other items). Packaging strapping bands were classified under the 'other plastic' category within this study, even though some authors have categorized them as originating from fishing activities because these items are known to be used in bait boxes (e.g., Cliff et al., 2002, Colmenero et al., 2017, Jepsen & De Bruyn, 2019). Our decision to classify them as 'other plastics' was based on the fact that packaging strapping bands are not associated with the gears used in fishing operations, then requiring different mitigation and remediation measures than ALDFG. Moreover, some may also originate from sources other than fisheries.

2.3.3. Colour classification

Following the recommendation of Provencher et al. (2017), we recorded the primary color of the entangling plastics. This is a characteristic often overlooked in studies of marine debris, yet it

might provide relevant information about the selectivity of marine animals in ingesting or becoming entangled in certain items. The classification was done by describing the items within eleven broad color categories (off/white-clear, blue, black, green, yellow, orange, red, brown, pink, grey/silver, and purple) (Provencher et al., 2017).

2.4. Physical status of the entangled animals

The injuries caused by entanglements were documented by initially classifying entangled animals as either 1) non-injured (highly active with no visible external injuries) or 2) injured (displaying external injuries or deceased). The lesions of live injured animals were further categorized as follows: a) minor (small scars, superficial scratches, skin abrasions, slight lacerations), b) moderate (significant wounds or cuts and injuries that are potentially recoverable), and c) severe (injuries such as flipper, fins or tail amputations, deep lacerations exposing significant damage, constrictions, body malformations, bone fractures, and malnutrition linked to increased drag, restrictions in mobility and/or impaired foraging). The primary body areas affected by entanglements were documented using a classification adapted from the INDICIT Consortium (2018) framework into: 1) upper body areas, including heads, necks, gills, bills, and beaks, 2) limb areas, including front or rear flippers, fins, tails, and wings, and 3) the mid-body area or the carapace. All analyses and graphs were performed using R (R Core Team, 2021).

3. Results

3.1. Overall results

A total of 95% (n = 39) of entangled animals were observed at sea, while 5% (n = 2) were found stranded along the coastline. Most entanglements were within the Azores EEZ (76%; n = 31), with a few (24%; n = 10) scattered records in the offshore east area of the EEZ toward the mainland (Fig. 6.1). Shark entanglements were recorded in the open ocean and near the islands (Fig. 6.1a). Entangled pelagic fish were observed exclusively in the open ocean. In contrast, cetaceans, sea turtles, and seabirds were only registered around the archipelago (Fig. 6.1b-c). The number of entanglement events in plastic debris varied between one and nine per year (Fig. 6.2a). On average, three animals (\pm SD = 2.56; \pm SE = 0.71) were found entangled per year between 2012 and 2024, with the majority being collected by fishery observers (44%; n = 18), followed by tourist operators (34%; n = 14), scientists (12%; n = 5), and the stranding network (10%; n = 4) (Fig. 6.2b). When grouped by taxon, sharks (\bar{x} = 1.42; \pm SD = 2.23; \pm SE = 0.65) and sea turtles (\bar{x} = 1.16; \pm SD = 1.34; \pm SE = 0.39) were the most frequently reported taxa per year.

Ten species from six families and five orders were found entangled (Table 6.1). Entanglements in non-fishing plastics were recorded in two bottlenose dolphins (*Tursiops truncatus*), fourteen blue sharks (*Prionace glauca*), and two swordfish (*Xiphias gladius*). In addition, entrapments in ALDFG included a minke whale (*Balaenoptera acutorostrata*), a sei whale (*Balaenoptera borealis*), a Bryde whale (*Balaenoptera edeni*), and a Sowerby's beaked whale (*Mesoplodon*

bidens), fourteen loggerhead turtles (*Caretta caretta*) and one green turtle (*Chelonia mydas*), three blue sharks and one Cory's shearwater (*Calonectris borealis*) (Table 6.1).

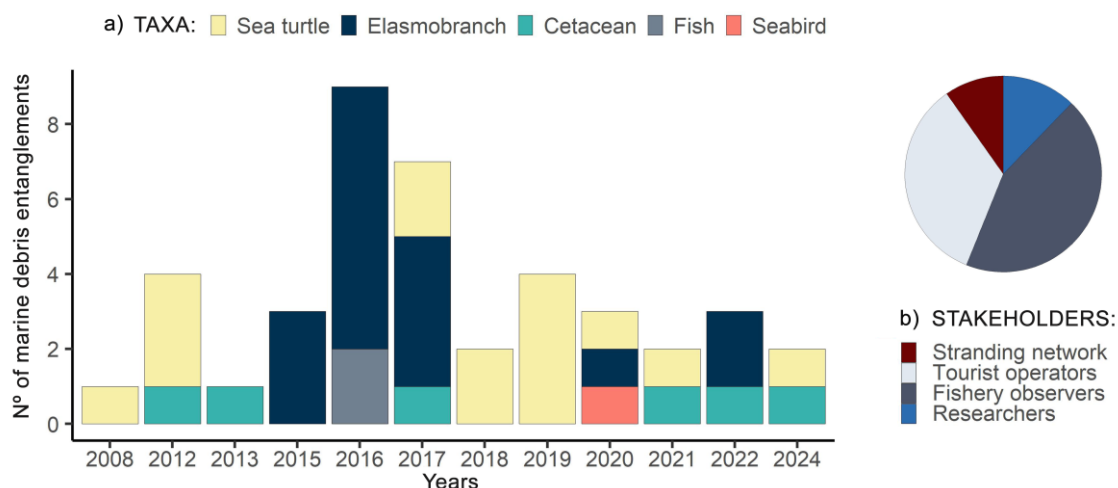


Figure 6.2. Frequency of animals found entangled in marine debris per a) taxa group and b) source of information. The data was collected between 2008 and 2024, but note that in the first graph (a), no events were included from 2009, 2010, 2011, 2014, and 2023.

Table 6.1. The orders, families, and species of documented animals entangled in plastic debris, the number and types of entanglements in the NE Atlantic.

Order	Family	Species	N° of entanglements (2008 - 2024)	Type of debris
Cetacea	Mysticeti	<i>Balaenoptera acutorostrata</i>	1	Fishing nets
		<i>Balaenoptera borealis</i>	1	Fishing nets
		<i>Balaenoptera edeni</i>	1	Ropes
	Odontoceti	<i>Tursiops truncatus</i>	2	Plastic bags
		<i>Mesoplodon bidens</i>	1	Fishing nets
Testudines	Cheloniidae	<i>Caretta caretta</i>	14	Fishing nets; conglomerate gears; ropes
		<i>Chelonia mydas</i>	1	Ropes
Carcharhiniformes	Carcharhinidae	<i>Prionace glauca</i>	17	Plastic straps; rings; fishing nets, ropes
Perciformes	Xiphiidae	<i>Xiphias gladius</i>	2	Plastic rings
Procellariiformes	Procellariidae	<i>Calonectris borealis</i>	1	Conglomerate gears

3.2. Characterization of the entangling debris

ALDFG accounted for half of the entangling items (56%; n = 23), of which 48% were fishing nets, 30% were conglomerates of fishing gear, and 22% were accessories such as ropes. Other plastics (44%; n = 18) consisted mostly of plastic strapping bands (67%), followed by hard plastic

rings (22%), and plastic bags (11%). Sea turtles and seabirds were found entangled exclusively in ALDFG (Fig. 6.3). Cetaceans were primarily found entangled in ALDFG (60%), though some cases involved SUPs (40%), with this taxa being the only group found entangled in plastic bags (Fig. 6.3). Most blue sharks (82%) and the two swordfish were recorded as entangled in other plastics. The only documented seabird was trapped in a conglomerate of different fishing gears, in which a loggerhead turtle was also found entangled but still alive. That conglomerate consisted of a small section of a trawl net and various types of ropes, some exhibiting signs of biofouling. Overall, the predominant colors of the entangling debris were green (39%; $n = 16$), off/white-clear (27%; $n = 11$), black (20%; $n = 8$), blue (7%; $n = 3$), orange (5%; $n = 2$) and brown (2%; $n = 1$).

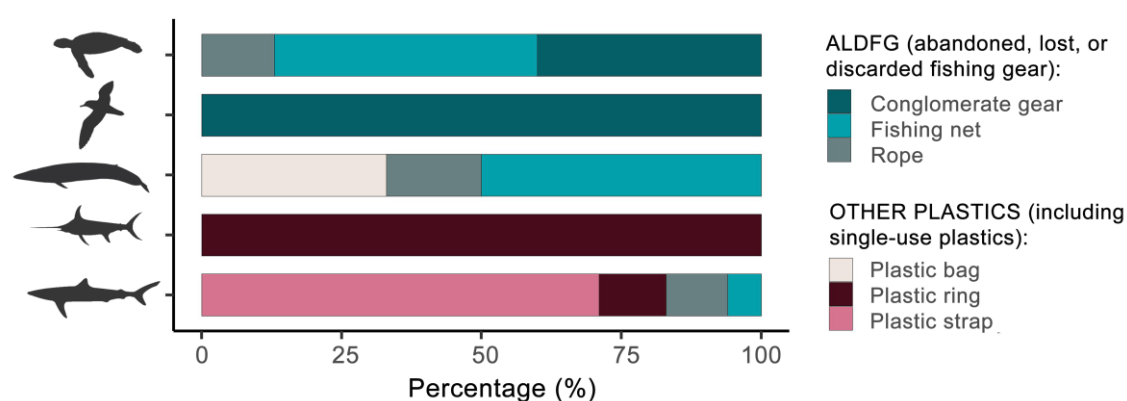


Figure 6.3. Typology of fishing-related entangling items affecting each of the taxa studied in the NE Atlantic.

3.3. Severity of the entanglements in marine debris

The majority of the entangled animals (78%; $n = 32$) had external body lesions (91%; $n = 29$) or were already deceased (9%; $n = 3$) as a result of entanglement in marine debris. Specifically, the injuries on the live animals were classified as 29% minor, 14% moderate, and 57% severe. Animals with no apparent injuries accounted for 17% of cases, while in 5%, the severity of injuries was impossible to assess. Detailed injuries in the live animals included 46% constrictions, 39% skin damage (including abrasion, wounds, lacerations, or cuts), 11% limb loss, and 4% body deformations. Fatalities were likely caused by drowning due to the increased drag produced by the entangling debris.

The most difficult taxa to determine the level of impact were the cetaceans, as the severity of potential injuries could not be determined in half of the cases due to the insufficient resolution of the available visual material. When the level of severity could be determined, we found that 17% of entangled cetaceans exhibited moderate damage and 33% were found to be severely injured. Cetaceans were mainly found entangled around the head, followed by the mid-body area.

All recorded entanglements of blue sharks affected the gill slit region and the insertion of the pectoral fins. The entangled sharks were juveniles (Fig. 6.4a-f), with 29% exhibiting minor

injuries, 12% moderate injuries, and 59% severe injuries. The swordfish were found with circular plastic debris identified as remnants of plastic cups around their bills. While one of the items had no biofouling (Fig. 6.4g), the second was fully covered by gooseneck barnacles (Fig. 6.4h-i). The sole seabird entangled in marine debris was found deceased, with the entangling debris (Table 6.1; Fig. 6.3) trapping its entire body.

Of all turtles found entangled, 33% were found uninjured and released from the entanglement on site, 54% presented injuries, and 13% were found deceased. Of the severely injured turtles, three had one of their forelimbs highly necrotic, ultimately leading to limb loss during recovery in captivity before being released. One of those examples involved a turtle trapped in a conglomerate of more than six types of ropes, with three colors and a total dry mass of 648.8g (Fig. 6.5). Moreover, one turtle exhibited carapace deformation, and four others had skin damage, such as abrasions or wounds.

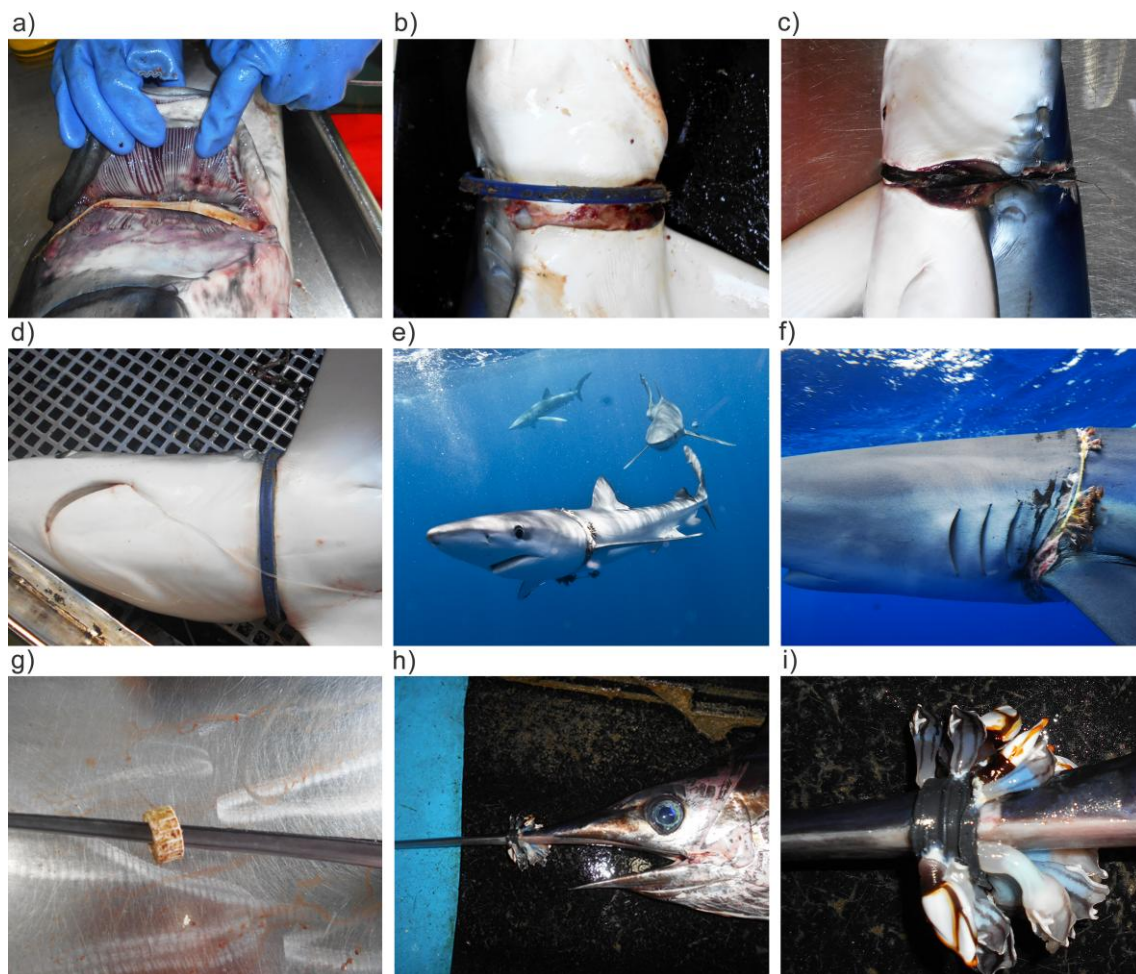


Figure 6.4. Sharks and bony fish entangled in other plastics, mainly single-use. a) A blue shark (*Prionace glauca*) with a white plastic strapping band around the gills; b) a blue shark entangled in a blue plastic ring with a visible wound; c) a blue shark entangled in a black plastic strapping band with a deep cut around the gills; d) a blue shark with a blue plastic ring entangled around the gill area causing constriction and visible wounds when the item was removed; e) a blue shark with a rope covered with biofouling around the gill area; f) detail of the entangling

item registered in previous picture; g) a swordfish with a plastic cup ring around the bill; h) a swordfish with a black plastic cup ring highly covered with barnacles; and i) close up of the entangling plastic of the previous picture. All these injuries were classified as moderate except for case *c*, classified as severe, and *h* and *i* as non-injured.

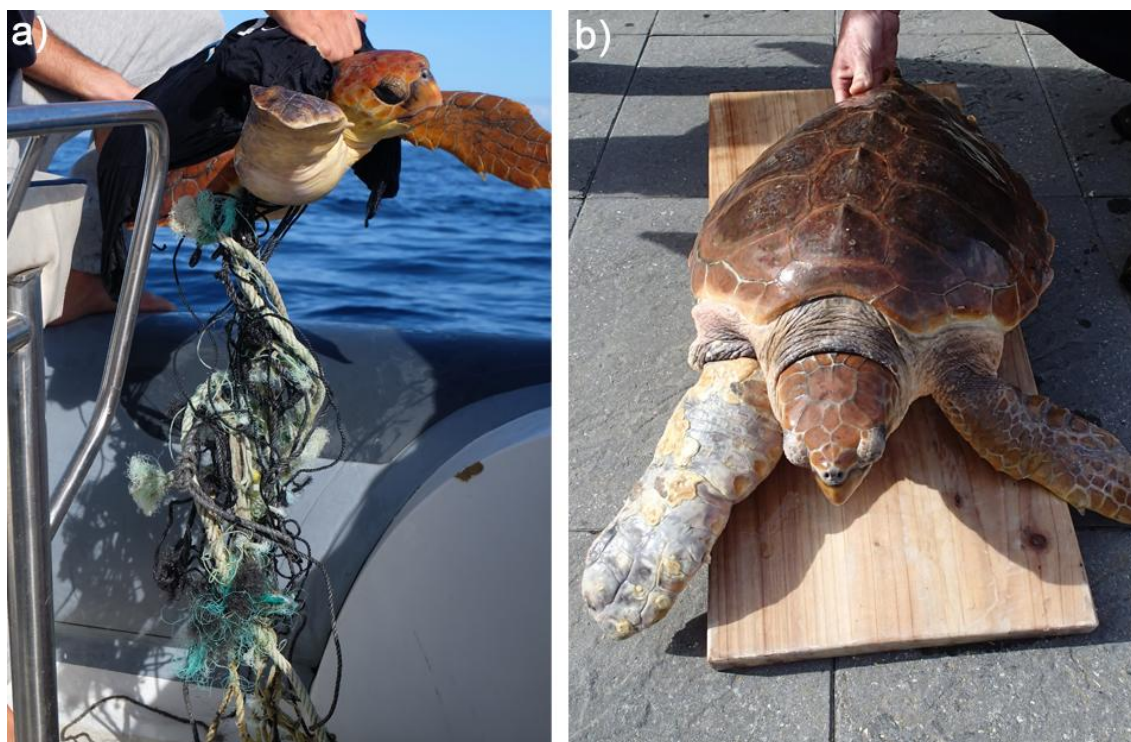


Figure 6.5. The case of a loggerhead turtle found entangled in ALDFG in 2018, whose injuries were classified as severe. a) The moment when the turtle was found; and b) the turtle upon removal of the entangling material with a visible constriction and subsequent swelling of the right front limb.

4. Discussion

This study presents the first comprehensive compilation of entanglement events in marine megafauna across an extended region of the NE Atlantic, integrating opportunistic data from diverse sources. Our study shows that entanglement in both ALDFGs and SUPs serves as an additional stressor, compounding the existing pressures that marine biota already face in the open ocean. Although we could not address sub-lethal effects, our findings indicate that entanglement in marine debris causes direct impact by inflicting external injuries (e.g., skin abrasions, lacerations, deep wounds, or cuts) and by constricting critical body areas (e.g., neck, gill slits, bills, beaks, limbs and carapaces), triggering in some cases limb loss or body deformations. These injuries may likely result in negative impacts on fitness (e.g., growth, predator vulnerability, fecundity), ultimately leading to mortality.

4.1. Sea turtles

The NE Atlantic provides critical foraging and developmental habitats for the early life stages of juvenile loggerhead turtles originating mainly from the Southeast USA and Cape Verde Islands (Bolten et al., 1993, Bjorndal et al., 2000, Bolten, 2003, Martins et al., 2018). Major

anthropogenic sources of sea turtle mortality in the region include fisheries bycatch (Parra et al., 2023) and, to an unknown extent, plastic ingestion and entanglement (Pham et al., 2017, Rodríguez et al., 2022). Previous studies suggest that life stage influences entanglement rates in this group. Juvenile pelagic sea turtles are usually more affected than adults due to their surface feeding behavior on floating prey and dependence on open-ocean environments, such as subtropical gyres normally associated with debris accumulations (Duncan et al., 2017, Senko et al., 2020). Our study documented several juvenile sea turtles entangled in fishing-related items, including ropes, fishing nets, and conglomerates of gears used in trawling. In other regions of the NE Atlantic where trawling is permitted, fishers are known to cut out parts and discard damaged trawl nets that need to be repaired (Metcalf et al., 2020, Metcalfe & Bentley, 2020). Although mitigation measures exist to encourage fishers to adopt best practices for net repair and handling, for example working in designated areas and following cuttings disposal protocols (Metcalf et al., 2020, Metcalfe & Bentley, 2020), discarded net fragments seem widely distributed and are frequently found as beached debris (e.g., Strietman et al., 2021), or on continental shelves and the seafloor (Pham et al., 2014).

In the Atlantic basin, there have been few studies reporting incidents of sea turtles entangled in marine debris. These studies have mostly concerned loggerhead turtles (e.g., Barreiros & Raykov, 2014, Orós et al., 2016), and to an even lesser extent, Olive Ridley turtles (Santos et al., 2012) and green turtles (Rodríguez et al., 2022). Studies from other regions have also reported a high incidence of trapped sea turtles in fishing-related items (e.g., Nicolau et al., 2016, Cantor et al., 2020, Hurtado-Pampín et al., 2024), but such studies did not differentiate between entanglements caused by operational fisheries and those resulting from marine debris.

In general, injuries caused by entanglements are more severe than those reported due to plastic ingestion in the same taxa (e.g., Archibald & James, 2018). For instance, in the Azores, 83% of loggerheads (Pham et al., 2017), 86% of green turtles (Rodríguez et al., 2022), and the only leatherback turtle recorded so far (Barreiros & Barcelos, 2001) were reported to ingest plastic. However, only one of these turtles has been documented to be negatively impacted by plastic ingestion (Pham et al., 2017). In contrast, we found that 78% of entangled turtles were severely injured in our study. Similarly, Barreiros & Raykov (2014) described two cases of loggerhead turtles in the Azores that lost their limbs likely due to entanglements in ALDFG. Sea turtles are resilient animals that can survive and live with lasting effects (i.e., the loss of a limb). Still, they may experience sub-lethal impacts (i.e., lower swimming efficiency and thus, reduced ability or increased energetic cost to feed, reproduce, or escape from predators), and even die after their release. The long-term survival of affected individuals is difficult to confirm without post-release monitoring (Duncan et al., 2017). Yet, one potential approach to addressing this knowledge gap

could involve tags combined with biologging sensors to evaluate impacts on feeding efficiency, energy expenditure, and key behaviors such as diving and swimming.

4.2. Marine mammals

Despite the fact that pinnipeds appear to be more susceptible to becoming entrapped in plastic debris (especially in packaging strapping bands; Nelms et al., 2021) than cetaceans (Simmonds, 2012), the number of cetaceans affected by this threat has increased (e.g., Baulch & Perry, 2014, Lusher et al., 2018). In part, the discrepancies in entanglement occurrences reported between these two groups may be attributed to the fact that pinnipeds are more easily studied than cetaceans because they can be observed in on-land colonies (Senko et al., 2020). Moreover, the elusive nature of cetaceans makes entanglements much more difficult to notice (Ramp et al., 2021).

The mid-Atlantic waters surrounding the Azores are recognized as an important habitat for a diverse range of cetaceans (Silva et al., 2014). Yet, we recorded only a low number of entangled animals from this taxon. This group included, nonetheless, both toothed whales and baleen whales, with the latter showing more species affected, contrary to findings from a literature review on debris interactions in the Northeastern Atlantic (James & Große, 2023). In our study, resident and migratory species were represented too. While our cetacean entanglement records were limited to areas around the islands, offshore regions near mainland Portugal have also been identified as high-risk debris zones for this group of animals (Sá et al., 2021).

Previous studies have reported that most of the entangling devices were found around the flukes of cetaceans (e.g., Wells et al., 1998, Jensen et al., 2009, Stelfox et al., 2016), though we mainly observed plastics entangled around the head region. Play behaviors involving plastic can act as a mechanism leading to entanglements and subsequent head constrictions in odontocetes (Rodríguez et al., 2023). In baleen whales, entanglement in debris can obstruct the baleen plates, hindering the animal's ability to filter prey from the water and potentially causing temporary or permanent damage that can disrupt feeding (Werth et al., 2024). Regarding the plastic typologies, most entanglements in cetaceans have been linked to ALDFG worldwide (e.g., Baulch & Perry, 2014, Lusher et al., 2018, Tulloch et al., 2020, Solomando et al., 2022, Shi et al., 2024), although the distinction between active fisheries and ALDFG entanglements is unclear in some of those studies. Notably, cetaceans entangled in other plastics, such as SUP debris, were also documented within our data.

4.3. Seabirds

Seabird entanglements have been underreported in scientific literature (Høiberg et al., 2022), probably because they are fragile marine vertebrates and smaller in size compared to other top predators affected by this threat. As a result, entanglements in this group may go unnoticed, as they often die far from shore, where they are easily decomposed, consumed, or sink (Laist, 1997).

Yet some of the earliest documented cases of entanglement in fishing-related litter date back to the 1980s, particularly in northern gannets *Sula bassana* (Schrey & Vauk, 1987). Increased monitoring efforts have focused on seabird breeding colonies that are more accessible (e.g., Kühn et al., 2015, Ryan, 2018). Seabirds are known to become entangled not only at sea but also in their nests when incorporating plastic litter as nesting material, which sometimes even results in the mortality of their chicks (e.g., Votier et al., 2011, Rodríguez et al., 2013). Juvenile seabirds have been considered more vulnerable to entanglements than adults, with cases involving non-fishing materials showing higher success of survival (Costa et al., 2020).

Entanglement in operational fishing gear (foul-hooking) affects many seabird species (Phillips et al., 2010), with approximately 44,000 birds bycaught or incidentally captured per year in monitored fisheries (Phillips et al., 2024). A recent study integrating data from a stranding network and rehabilitation center in Portugal could not distinguish whether seabird entanglements were caused by marine debris or bycatch. Both threats were included under the same classification, affecting 42% of all animals in this group (Costa et al., 2021). In our study, only one Cory's shearwater was entangled in marine debris. On the contrary, the same species have been reported highly affected by bycatch in other Atlantic and Mediterranean regions (e.g., Báez et al., 2014, Calado et al., 2021). The low number of entanglements in marine debris detected in Cory's shearwaters in our study contrasts with their high incidence of plastic ingestion in the same region, which surpasses 90% in a sample of more than 1,000 birds (Rodríguez et al., 2024).

4.4. Elasmobranchs and fish

Elasmobranchs appear to be most vulnerable to ALDFG (Parton et al., 2019), but other plastics unrelated to fishing activities, such as polypropylene strapping bands, also impact this group (e.g., Cliff et al., 2002, Colmenero et al., 2017, Barreto et al., 2019). Elasmobranch entanglements have been reported to occur predominantly in the gill region, while the mouth, dorsal, and caudal areas are less commonly affected (Parton et al., 2019). The juvenile blue sharks documented herein were exclusively entangled in the gill slits area, exhibiting visible injuries, such as wounds and lacerations. As these juveniles grow, entanglements in the gill slits are expected to cause severe constrictions. This is likely to impair ventilation efficiency, with unknown long-term effects on their fitness, ultimately potentially leading to mortality. Blue shark entanglements in circular items similar to the ones observed in our research, such as plastic strapping bands or hard plastic rings, have been reported in the Indian and Atlantic Oceans (Cliff et al., 2002, Colmenero et al., 2017, Barreto et al., 2019). Similarly, tiger sharks (*Galeocerdo cuvier*) have also been found with similar typologies of entangling items around the gill area in the western South Atlantic Ocean (Afonso & Fidelis, 2023).

Remarkably, in our study, entangled sharks were not only documented by the fishery observers but also by scientists and sea users such as diving companies. This finding supports the hypothesis that these occurrences may be more ubiquitous than expected. Conversely, no information was found in the scientific literature about swordfish with hard plastic rings lodged around their bill, as observed in this research. The significant accumulation of biofouling covering entangling plastics found on their bills is likely to impact the animals' hydrodynamics, increasing the cost of transport and limiting their hunting efficiency. However, the underlying causes of these entanglements around the anterior body region are still unclear in both groups. Currently, no evidence of play behaviors could end up in subsequent SUP entanglements in sharks or even fish, contrary to what happens with cetaceans (Rodríguez et al., 2023) and potentially pinnipeds (e.g., Hanni & Pyle, 2000, Jepsen and De Bruyn, 2019), from which otariids may also suffer neck collars entanglements from artisanal and recreational fishing gear due to their foraging behavior (Franco-Trecu et al., 2017). Yet, blue sharks have occasionally been observed inspecting marine litter using their snout (Fontes, personal observation, 2024), a behavior that may easily increase the chances of entanglement in circular-shaped objects. In billfish, empirical studies have shown that bills serve as predatory tools with species-specific hunting strategies. However, no support for sensory functions exists, suggesting that billfish entanglements could be related to foraging (Domenici et al., 2014, Hansen et al., 2020).

4.5. Plastic typologies causing the entanglements

Globally, most studies identified ALDFG as the predominant entangling debris affecting megafauna (e.g., Duncan et al., 2017, Parton et al., 2019). Although this pattern was also observed in our study, after applying the ENSA index (Rodríguez et al., 2025), we found that some animals were likely entangled while the gear was still operational. As a result, both ALDFG and the other plastics category had, at the end, similar entanglement frequencies. Specifically, the non-fishing plastic category documented herein consisted exclusively of SUPs (i.e., plastic strapping bands, hard plastic rings, or handles of plastic bags). While we only observed blue sharks, swordfish, and cetaceans trapped in circular plastic items, seabirds have also been documented with plastic bottle rings entangled around their heads or beaks in other regions (e.g., Nisanth & Kuma, 2019).

4.6. Importance of monitoring entanglements in marine debris

Plastic pollution is a global issue impacting highly mobile marine megafauna (Nelms et al., 2023), with surface plastic debris levels in vast ocean regions, such as the North Atlantic, affecting several pelagic species (Høiberg et al., 2022). Consequently, collaboration among stakeholders within a well-organized network can be a valuable approach to gathering reliable data. A standardized and systematic survey by researchers of megafauna entanglements in marine debris is time-consuming and costly, as demonstrated by the discrepancy between the number of events reported on media platforms versus those documented in scientific literature (Nelms et al., 2023).

For instance, in our study, only 10% of the entanglement records reported by sea users were collected by scientists, and no research group has dedicated an at-sea survey to monitor entanglements. Moreover, the effort (time at sea) involved in commercial activities is significantly greater than that of scientific campaigns, especially when multiple companies run several trips per day. In this context, a diverse stakeholder contribution was crucial in this study, and thus, a broader engagement would be important in future monitoring.

The limited time dedicated to collecting data at sea, combined with frequent adverse weather conditions in open ocean areas, suggests that the number of entangled animals and the severity of this threat in the region may be underestimated. An additional cause of underreporting is the quality of certain data. More than sixty records collected since 1997 consisted solely of brief notes without pictures or videos, and even when visual material was available, its quality was often inadequate due to factors such as distance, blurriness, or lack of underwater visibility. This constraint excluded several potential cases of entanglements in marine debris from our study. Finally, because the dataset is opportunistic, variability in reporting effort is influencing entanglement patterns. For example, the apparent increase in events between 2016 and 2017 should be viewed cautiously, as it results from greater effort by fisheries observers rather than a real rise in occurrence. Likewise, reduced observer effort or fewer trips by other sea users could yield the opposite trend. Therefore, future analyses of spatio-temporal trends should account for sampling effort to ensure accurate interpretation.

Overall, the information provided in this baseline study will assist in identifying issues in data collection, helping to improve and optimize the process in the future. Clear guidelines for stakeholders to accurately report entanglement events in megafauna are essential to ensure data quality, support long-term monitoring, and inform effective policy actions. These guidelines should address not only the collection of imagery but also the recording of key contextual data such as the species affected, geographic location, and other relevant characteristics. This information will be critical for assessing Descriptor 10 (D10) Criteria 4 (C4) of the European Marine Strategy Framework Directive (MSFD). Specifically, the D10C4 requires EU Member States to quantify the number of individuals adversely affected by entanglements in debris, and its associated health-related effects (e.g., European Commission, 2008, Korpinen & Braeger, 2013, European Commission, 2017).

5. Conclusion

Even though opportunistic reports may suffer from several potential biases and are likely to underestimate the true magnitude of the problem, they can substantially increase our knowledge of a threat that is challenging to detect during dedicated field campaigns (GESAMP, 2019). In that sense, our study contributes to documenting underrepresented megafauna entanglements in marine debris across open-ocean regions of the NE Atlantic, indicating an average of at least three

pelagic vertebrates being found entangled in marine debris annually within the area covered. This is particularly concerning as the region studied serves as a crucial habitat for various migratory and resident marine vertebrates, including several juvenile phases (Vandeperre et al., 2014b, Vandeperre et al., 2019, Afonso et al., 2020). The species documented entangled in marine debris herein (blue sharks, swordfish, loggerhead turtles, bottlenose dolphins, Cory's shearwaters, green turtles, minke whales, Bryde whales, Sowerby's beaked whales, and sei whales) are inherently vulnerable to anthropogenic impacts due to their high longevity and low fecundity rates. Moreover, all of them are either endangered, threatened, or otherwise protected under international conservation agreements or regulations such as the IUCN Red List of Threatened Species and the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES). This highlights the importance of careful monitoring and management of human pressures, such as those caused by marine litter. Overall, this research reinforces the notion that the conspicuous presence of floating plastic has turned the open ocean into a hazardous environment, underlying the necessity to further investigate the sub-lethal effects and long-term population impacts of this threat for marine megafauna.

CHAPTER VII

General Discussion

Since the 1960s, studies have revealed that plastic ingestion is widespread among marine organisms, affecting even animals in the most remote environments on Earth (e.g., Bessa et al., 2019a; keo, 2023; Lenoble et al., 2024; Thompson et al., 2024). Likewise, in the last decades, entanglement in marine debris has emerged as a significant threat to wildlife (e.g., Duncan et al., 2017; Parton et al., 2019). These mounting pressures have promoted global efforts to assess and mitigate the impact of marine plastics through scientific research and policy interventions. However, the complexities of tracking such a dynamic pollutant, alongside the challenge of studying species as elusive as large marine megafauna, have resulted in a limited understanding. To truly grasp the spatio-temporal trends, sources, accumulation patterns, associated pollutants, and ecological consequences of ocean plastics, further research has been essential. The body of work presented in this dissertation seeks to address and narrow some of these lacunae, improving our knowledge of this pressing issue.

This thesis advances our understanding of plastic ingestion in a seabird species, demonstrating its role as a bioindicator (Chapters II and III). Chapter II provides valuable insights for policy-makers on monitoring floating plastics in an area that lacks an effective official programme. Using Cory's shearwater fledglings as indicators can help establish the EU-Macaronesia archipelagos as key observatories of North Atlantic plastic dynamics. Expanding this approach to similar shearwater species could further enhance global assessments of marine plastic pollution. Additionally, this thesis also documents in Chapter IV a lesser-understood behaviour – the playful interactions of cetaceans with plastic litter. While play is a natural behavior that supports an animal's socio-cognitive development, we have documented that play with single-use plastics is widespread and may lead to harmful consequences. Simultaneously, the Entanglement Source Assessment (ENSA) index developed within Chapter V addresses a global necessity. This is primarily because identifying sources of entanglements in fishing-related debris has relied so far on an expert opinion rather than a systematic approach. ENSA now provides a tool that can support a more transparent classification of those events. Finally, Chapter VI addresses the first comprehensive analysis of entanglements in marine debris across diverse taxa throughout the NE Atlantic, a threat generally underreported in the current literature. The results showed that entanglements in both ALDFG and SUPs act as an additional anthropogenic stressor in the open ocean, affecting several groups of megafauna already threatened by numerous other human pressures.

7.1. Advances in the use of bioindicators for monitoring plastic pollution

Despite international policies calling for bioindicators to monitor plastic contamination in the marine environment, only a few programs have been implemented worldwide (Savoca et al., 2025). Due to the susceptibility of Procellariiform seabirds to plastic contamination, different species have been proposed as promising candidates (e.g., van Franeker et al., 2011; Phillips and Waluda, 2020; Lopes et al., 2022; Lavers and Bond, 2024). Their large populations, vast geographical distribution, and easy accessibility at their colonies, along with their foraging strategy, exclusively at sea, make them suitable for this role (e.g., Roman et al., 2019a; Roman et al., 2020; Nelms et al., 2023). In the NE Atlantic, the northern fulmar is the most established bioindicator for monitoring plastic pollution (e.g., van Franeker et al., 2011; OSPAR, 2015; van Franeker et al., 2021). However, despite their significance, northern fulmars are absent from southern EU countries, including those located in the Macaronesia region. This gap has driven policy and research efforts to identify an alternative species that could provide adequate responses to international directives. In light of this, Chapter II investigates plastic ingestion in a large number of dead Cory's shearwaters collected in the Azores, surpassing 1,000 individuals. Data obtained from those birds were analysed in combination with samples from the Canary Islands.

The Azores are a vital breeding ground for Cory's shearwater (*Calonectris borealis*) (Monteiro et al., 1996a). Therefore, to protect the fledglings disoriented by artificial light pollution, the Azorean Government launched the SOS Cagarro rescue campaign in 1995. Despite significant efforts to limit casualties, not all rescued birds survive. Therefore, the programme allows for the collection of a decent number of deceased individuals across most islands, making them available for scientific purposes, including studies on plastic ingestion. In this context, Chapter II provided the first data on plastic ingestion in a seabird species breeding in the Azores. It revealed that Cory's shearwaters largely ingest small hard plastic fragments, which differ in morphology from the ones mainly ingested by the Canary nesting population. The thesis reinforces that the primary source of contamination in these other birds is largely influenced by fishing-related debris from the West African coast, as demonstrated in previous (Rodríguez et al., 2012a; Navarro et al., 2023) and more recent studies (Sobrino-Monteliu et al., 2025).

Similar, though smaller, rescue campaigns for Cory's shearwaters also exist in other parts of Macaronesia, including Madeira Island and the Canary Islands (Atchoi et al., 2021), allowing the implementation of this bioindicator in these regions. Moreover, Chapter II is notable as it is one of the few studies to address the challenge of establishing a threshold value for evaluating Good Environmental Status (GES) to support the reporting of floating plastic-related data to the MSFD and OSPAR. It also presents an innovation, particularly in the plastic metric selected for the threshold. While the northern fulmar has traditionally used plastic mass as a reference to monitor the abundance and temporal trends of this contaminant in the marine environment (e.g., van

Franeker et al., 2011; van Franeker et al., 2021), findings in Chapter II indicate that, in contrast, the number of plastic items is the stable and reliable metric for the same goal when using Cory's shearwaters (Fig. 7.1).

Chapter II also defies the existing literature by establishing the indicator exclusively based on data collected from fledglings. In contrast, other bioindicator assessments often rely on heterogeneous samples (e.g., Galgani et al., 2022). Regarding the northern fulmar, some studies suggest that including birds of varying ages, collected through different methods (i.e., bycatch, stranded, hunted or affected by other pollutants such as light pollution) or across different seasons, may not lead to significant bias in bioindicator assessments if these factors are properly accounted for in the analysis (e.g., van Franeker and Meijboom, 2002; van Franeker et al., 2021; van Franeker et al., 2022; Kühn et al., 2022). However, it is generally recognized that such variables could potentially influence the results, and careful consideration is needed to ensure the accuracy and reliability of the bioindicator data. For instance, birds of different ages may ingest different amounts or types of plastic (e.g., Tulatiz et al., 2023), and collection methods (e.g., Rodríguez et al., 2018) or seasons (e.g., Ryan, 2015) can influence plastic loads too.

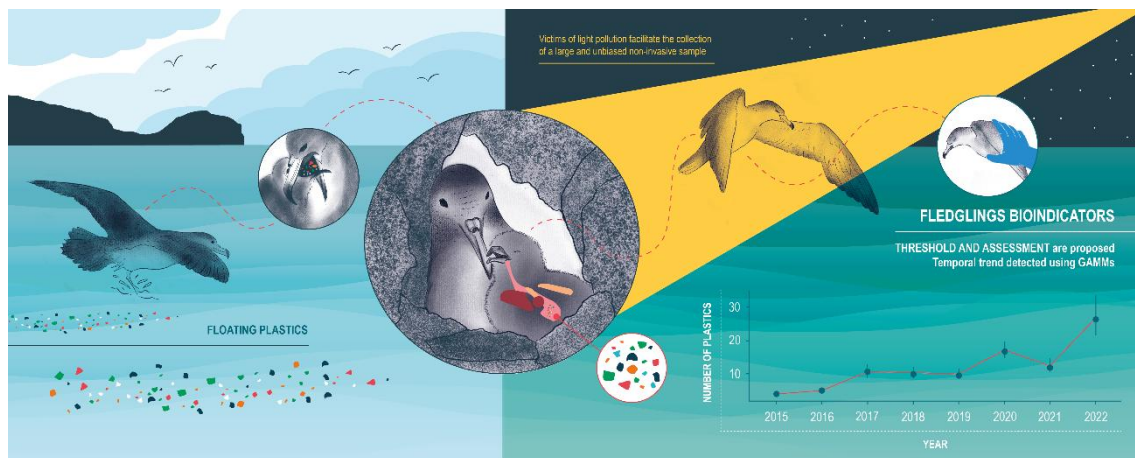


Fig. 7.1. Graphical abstract from Rodríguez et al. 2024 (chapter II).

The intergenerational transfer of plastics is clearly illustrated in Chapter II, where sampling of both fledglings and adults revealed notable differences in the frequency of plastic ingestion between the two age groups (93%FO and 59%FO, respectively). While previous studies on shearwaters (Carey, 2011; Rodríguez et al., 2012a) have indirectly suggested parental transfer of plastics to chicks, our further investigation in Chapter III uncovered that adults experience a reduction in plastic concentrations during chick-rearing. This chapter emphasizes the significance of this reduction as a ‘plastic reset’. It also highlights that including adults during certain periods of their annual biological cycle could introduce a confounding factor, potentially affecting the reliability of the results (Fig. 7.2). The fact that Chapter III documents those breeders to reset their plastic loads post-breeding suggests that, unlike fledglings, adults are less reliable indicators of plastic contamination. The decrease in plastic concentrations previously observed in adult

seabirds during breeding (e.g., Van Franeker and Meijboom, 2002; Skira, 1986; Van Franeker and Bell, 1988; Mallory, 2008; Provencher et al., 2010; Ryan, 2015; Nono-Almeida et al., 2023; Tulatz et al., 2023) has led to several other hypotheses to explain this phenomenon. Some of those studies have largely overlooked that plastic concentrations in adult birds may be influenced by their nesting cycle (e.g., Ryan, 2015). In consequence, Chapter III challenges existing literature in this regard and further reinforces the reliability of fledglings as the most accurate age to be implemented as bioindicators. Additionally, migratory seabirds are unreliable bioindicators at the start of the breeding season, as they may carry plastics from distant winter foraging areas. In the case of Cory's shearwaters, those plastics likely originate from the South Atlantic, far from the EU-Macaronesia region (Dias et al., 2019).

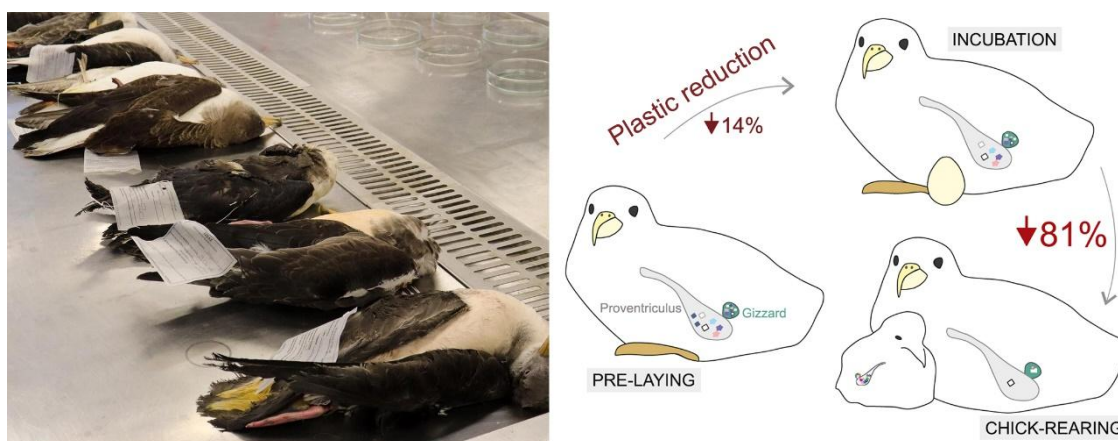


Fig. 7.2. Picture taken during necropsies and graphical abstract from Rodríguez et al. 2025a (Chapter III).

7.2. Fulfilling gaps about entanglement and other interactions with litter

Due to the limited information available on entanglements in the marine environment, and building on a previous study involving green turtles in the region (Rodríguez Y. et al., 2022), an idea emerged to explore this other topic further, involving information from different stakeholders and animal groups. Yet, during this investigation, two new questions emerged. On one hand, some of the photographic material received depicted cetaceans with plastic on various parts of their bodies, though they did not appear to be entangled. The accompanying notes consistently mentioned the word 'play'. This sparked a curiosity to investigate whether such behaviour was possible, if it had been documented in scientific literature, and whether it could have any consequences for the animals.

Drawing from the information gathered in the Azores, we expanded our research globally. Despite the widespread presence of plastic debris in marine environments, reports of playful interactions with plastic litter remained scarce. Only a handful of brief notes exist in scientific literature (Kuczaj and Highfill, 2005; Ii and Yeater, 2007; Di-Méglio and Campana, 2017). However, these

studies did not focus specifically on play behaviour with plastic, and before Chapter IV, no dedicated research was conducted to explore this interaction in depth.

Chapter IV reports that various cetacean species engage in play with single-use plastics, not only in the Azores region but across nearly all oceans, a behaviour observed so far in at least eleven different species of odontocetes (Chapter IV; Fig. 7.3). Chapter IV documented that play interaction occurs primarily with single-use plastics (SUPs). The packaging industry that generates SUPs worldwide is the largest plastic production and consumption market, representing around 44% of plastic demand in our society (Plastic Europe, 2022). This has been driven primarily by a global shift from reusable materials, particularly in food container applications, toward the widespread adoption of single-use containers where sustainable packaging is limited (Phelan et al., 2022). This trend has profound implications for environmental sustainability, as single-use plastics contribute to global waste generation and pollution challenges (Petrenko et al., 2024). While playing behaviours between marine mammals and SUPs may appear harmless, Chapter IV suggests that those interactions may ultimately lead to the ingestion of plastics or entanglement. Environmental responsibility, social and legislative influence, enforcement, compliance, material preferences, personal guilt, and income are key factors shaping willingness to adopt a SUP ban, highlighting the need for a multifaceted approach in policymaking to tackle this problem (Suhardono et al., 2025).

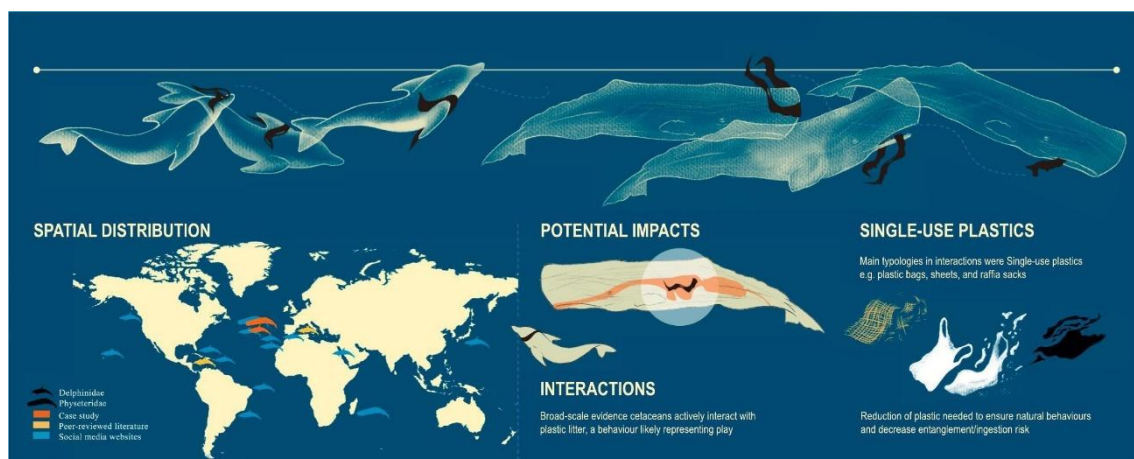


Fig. 7.3. Graphical abstract from Rodríguez et al. 2023 (Chapter IV).

While the sources of single-use plastics (SUP) in marine litter are easily identifiable, allowing for concrete management actions (e.g., Directive EU 2019/904), the classification of entanglements in fishing-related items is more complex. So, while analysing the rest of the entanglement events received by the various stakeholders, we asked ourselves another question: can we be certain that the entanglement information we received involving fishing-related items was all caused by marine litter? Determining whether certain entanglements stem from an operational fishery at the time of the event or, in contrast, from an ALDFG that is already part of marine litter, has often relied on subjective expert opinions rather than a systematic approach. To address this, Chapter

V (Fig. 7.4) improves the accuracy of reporting fishing-related item entanglements. This is relevant because some studies have already highlighted this need (e.g., Asmutis-Silvia et al., 2016; Richardson et al., 2019), as other researchers have misattributed the source of entanglement in fishing-related items (Stelfox et al., 2016). Hence, the Entanglement Source Assessment (ENSA) index developed within this thesis provides a transparent and standardised tool that, through various criteria, enhances our ability to classify entanglement events between OFG and ALDFG. By developing a standardised classification system for these events, we expect that the method will be applied in other regions. This will ensure that data repositories dedicated to entanglement in ALDFG (or its counterpart, OFG) exclusively contain entanglement events caused by each of the threats. This will support better comparison between the roles of OFG vs. ALDFG in the entanglement of marine organisms, facilitating targeted strategies for battling each pressure. Indeed, distinguishing between these two threats is crucial since the mitigation measures for each source differ significantly (Williams and Rangel-Buitrago, 2019; Bellou et al., 2021; García et al., 2024; Villafáfila et al., 2024), which can lead to public and policy misinterpretation that misallocates resources away from the most urgent.

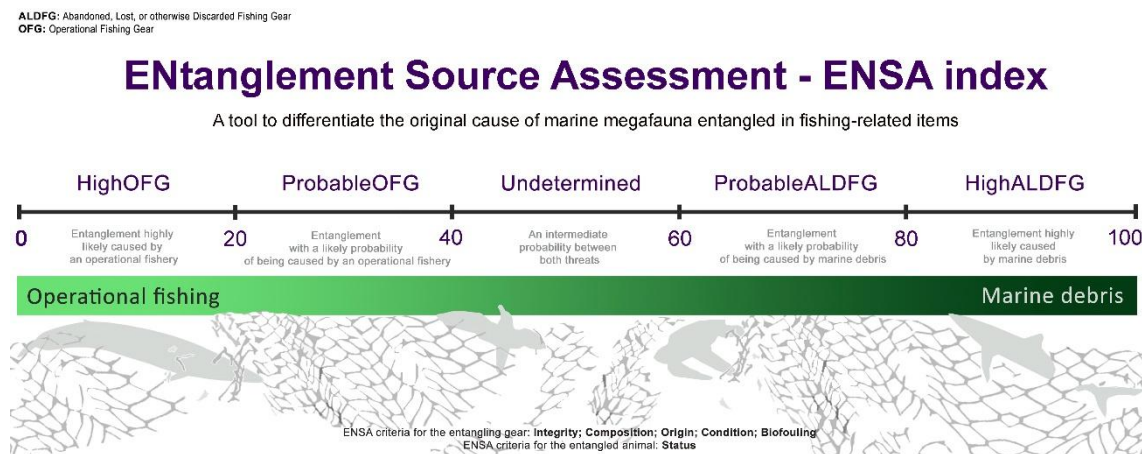


Fig. 7.4. Graphical abstract from Rodríguez et al. 2025b (Chapter V).

After answering the two previous questions, and thus removing play behaviours and those influenced by operational fishing, we finally compiled a clean and accurate database of entanglement events in marine litter. This allowed us to document the most frequent entangling items, species affected, and impacts. Chapter VI highlights a largely overlooked threat posed by marine litter due to the challenges of collecting reliable, high-quality data on this issue.

Studies, particularly addressing complex and diffuse threats, like marine litter entanglements, should involve diverse stakeholders (e.g., fishers, conservationists, policymakers, citizen scientists, and local communities). These stakeholders often provide crucial data that would be difficult or impossible to collect otherwise. For instance, by integrating information from fishery observers onboard pelagic longline fishing vessels, we were able in Chapter VI to include information from waters off mainland Portugal, covering a broader area in the Northeast Atlantic.

Overall, the three studies developed in the second part of the thesis reinforce the importance of multidisciplinary collaboration in marine conservation research.

7.3. Thesis information feeding into policy

Before this thesis, there was limited scientific information available to support the Portuguese Government in reporting to the MSFD D10C3 and D10C4. Specifically, the outcome of Chapter II contributed information for the 2025 MSFD report. Moreover, the findings from Chapter II and Chapter III supported the promotion of Cory's shearwater fledglings as an official indicator for monitoring floating plastics in the NE Atlantic for the OSPAR Commission (Annexe I). This proposal, led by the Azores Government, has been submitted to the OSPAR committee for approval at the Environmental Impact of Human Activities (EIHA) meeting scheduled for April 2025. The new tool (ENSA – Entanglement Source Assessment Index) developed in Chapter V was designed to enhance the reporting of the source of entanglement events on fishing-related gear for the implementation of better management actions to mitigate each pressure (operational fishing vs. marine litter). ENSA has already been considered within an OSPAR background document on entanglement promoted by the Spanish Government (for discussion at the EIHA meeting in April 2025). Thereby, offering support for policy implementation at both local and international levels. While the other chapters of this thesis have not yet received strong political attention, they will also contribute to future developments. Chapter VI will serve as a baseline for a more systematic data collection for the next MSFD D10C4 report. Chapter IV may reinforce the need for policies within the EU to battle single-use plastics at different levels, and it has already been cited in studies aiming to ban single-use plastics in other regions (e.g., Suhardono et al., 2025).

7.4. Future research

Despite the achievements attained during this thesis, future research is still needed within its scope. For example, it would be essential to explore Cory's shearwater pathway of plastic ingestion to determine whether they actively select plastics, inadvertently ingest them, or if some plastic sizes are secondarily transferred through their diet. Although this is a challenging study, it is crucial to understand the underlying mechanisms of plastic ingestion and ensure that Cory's shearwater effectively monitors what is available in the environment. This study could combine plankton trawls to collect visible plastics with stomach analyses of their typical prey items. We can also expand our understanding of plastic ingestion pathways through innovative methods, such as utilising cameras attached to the animals to detect encounters with plastics during foraging. Moreover, analysing plastic ingestion in other seabird species breeding in the Azores could offer valuable comparisons with Cory's shearwaters and provide insights into the underlying causes of their plastic burden. If we observe that different species with vastly different foraging behaviours ingest the same types of plastics, this may suggest that what Cory's

shearwaters consume reflect the composition and abundance of plastics in the environment. This will indicate that ingested plastic is not the result of a selection process based on items that most closely resemble their natural diet, which would otherwise limit the bioindicator role and the environmental conclusion about this contaminant taken from it.

It would also be valuable to gain a deeper understanding of the presence of microplastics smaller than 1 mm in the birds' gastrointestinal (GI) tracts. This will be important not only by analysing stomach contents, but also by extending the investigation to the intestines (Bessa et al., 2019b). The presence of microplastics of any size can impact seabird health (e.g., Charlton-Howard et al., 2023; Fackelmann et al., 2023; de Jersey et al., 2025), making it crucial to investigate the total content of plastics in the GI. Additionally, there is limited information on whether plastics remain in the gizzard until they degrade. The study of small microplastics could help determine whether they result from ingestion, such as through diet or water, and/or whether they result from the breakup of larger items in the gastrointestinal tract. It is also crucial to assess whether Cory's can serve as a bioindicator for plastics smaller than 1 mm, as such indicators are necessary but have not yet been widely implemented.

Gaining a better understanding of the size threshold at which plastics are excreted could significantly contribute to our knowledge of plastic retention times. Retention time is the period plastics remain in a seabird's digestive system before being expelled or degraded, which is also a relevant factor for the bioindicator assessments. Seabirds also transport pollutants to pristine regions (e.g., Blais et al., 2005), acting as sinks for plastic debris within their breeding colonies by carrying plastic from marine to terrestrial environments (e.g., Provencher et al., 2018; Grant et al., 2021). Studying retention times will help assess whether plastics act as vectors of microplastics between environments. Moreover, investigating the relationship between the ingestion of plastics and the accumulation of persistent organic pollutants (POPs) in Cory's shearwaters may contribute valuable data to the scientific literature and the role of this species as a bioindicator. POPs can be investigated in different matrices (environmental plastics and seabird tissues) to assess whether plastics act as a vector for transporting and transferring these compounds to biota.

While this thesis offered significant advances in marine megafauna entanglement in marine debris, more can be done to improve our understanding. With a larger entanglement dataset collected in the Azores, we can determine with higher certainty whether events in fishing-related items occur more frequently in OFG than ALDFG. In the future, it would be important to apply the ENSA index to other regions around the world. This will help determine its effectiveness on a global scale, allowing for comprehensive assessments of fishing-related entanglements and providing valuable insights for policymakers. Moreover, strengthening partnerships with stakeholders such as tourist operators, diving companies, fishery observers, and stranding

networks is essential for efficient data collection, requiring a well-harmonised protocol and strengthened partnerships. This would transform data collection from an opportunistic approach to a more systematic and detailed framework, improving future estimates of event frequency of entanglements in marine litter in the NE Atlantic. It would also provide more data on the most affected species, the predominant types of debris, and their health consequences. This information will provide stronger support for reporting such events to the D10C4 of the MSFD. In that way, enabling more effective measures focused on the most common litter types affecting specific taxa. Moreover, it will be essential to monitor animals that, for example, have recovered from severe injuries resulting from entanglements through tags and biologging sensors. This would help to understand the additional impacts or sub-lethal effects arising from this threat. Lastly, identifying other interactions with plastics, such as play behaviour, should be continually monitored to systematically detect their occurrence, the species involved, and to have a more accurate idea of the impacts of these interactions. In addition, with clear protocols and informed stakeholders, it will be possible to determine if other groups of marine vertebrates, such as sharks or fish, also experience entanglements in the upper part of their bodies (i.e., gills or beaks) driven by curiosity rather than feeding-related behaviours.

Final Conclusion

To sum up, this thesis underscores the need for multifaceted approaches to better understand the impact of plastic pollution on marine vertebrates. It provides valuable insights for policymakers to develop targeted strategies that improve reporting and compliance with environmental policies. The scientific chapters developed within this thesis have reinforced that, for marine top predators, plastic is an ever-present hazard entering their bodies through ingestion or impacting them through entanglements. The human-driven challenges of establishing an effective global treaty to tackle plastic pollution necessitate a continuous and deeper understanding of this pressure, particularly in the oceans, where plastics persist and accumulate in complex ways. In particular, research on its impacts on marine vertebrates enhances our knowledge of this anthropogenic threat in species inherently vulnerable due to their long lifespans and low fertility rates, and provides crucial insights for informed decision-making. Such efforts underscore the urgency of addressing marine litter pollution through stronger legislation, increased political pressure, and greater public awareness. Ocean plastics are a vast and intricate human pressure, stretching across borders and sectors, with profound ecological, social, political, and economic repercussions. Overall, we hope this thesis will serve as a small but meaningful step toward enhancing scientific knowledge and driving meaningful conservation efforts to protect vertebrates, including marine megafauna, from the growing dangers of the Anthropocene.

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Appendix A – Supplementary Material chapter II

Source: Rodríguez, Y., Rodríguez, A., van Loon, W.M., Pereira, J.M., Frias, J., Duncan, E.M., Garcia, S., Herrera, L., Marqués, C., Neves, V. and Domínguez-Hernández, C., 2024. Cory's shearwater as a key bioindicator for monitoring floating plastics. *Environment International*, 186, p.108595. <https://doi.org/10.1016/j.envint.2024.108595>

Table S2.1. Overall number of samples from adults and fledglings of Cory's shearwaters (*C. borealis*) across the two breeding areas (the Azores and the Canary Islands) and years of the study.

N° of Cory's shearwaters			
Years	Azores archipelago	Canary Islands	Total
2015	149	18	167
2016	168	65	233
2017	100	24	124
2018	125	0	125
2019	193	0	193
2020	171	0	171
2021	165	0	165
2022	60	0	60
Total	1,131	107	1,238

Table S2.2. Number and mass of plastic ingested (zeros included) by Cory's shearwaters fledglings (a) and adults (b). The reporting metrics are the arithmetic mean (mean), the standard deviation (\pm SD), and the standard error (\pm SE).

a) Fledglings of Cory's shearwaters

Year	Region	N	N° of plastics			Mass of plastics (g)		
			Mean	SD	SE	Mean	SD	SE
2015	Azores	148	4.1	4.0	0.3	0.0178	0.0263	0.0022
2016	Azores	160	4.9	4.2	0.3	0.0185	0.0246	0.0019
2017	Azores	96	11.0	7.9	0.8	0.0243	0.0317	0.0032
2018	Azores	116	11.9	19.0	1.8	0.0167	0.0184	0.0017
2019	Azores	155	9.9	8.2	0.7	0.0211	0.0334	0.0027
2020	Azores	162	17.1	13.0	1.0	0.0237	0.0228	0.0018
2021	Azores	142	11.7	9.3	0.8	0.0117	0.0151	0.0013
2022	Azores	51	25.9	18.5	2.6	0.0242	0.0180	0.0025
2015	Canaries	18	37.0	20.9	4.9	0.0307	0.0206	0.0049
2016	Canaries	57	34.6	22.6	3.0	0.0394	0.0285	0.0065
2017	Canaries	22	5.3	3.3	0.7	0.0101	0.0097	0.0021

b) Adults Cory's shearwaters

Year	Region	N	N° of plastics			Mass of plastics (g)		
			Mean	SD	SE	Mean	SD	SE
2015	Azores	1	0.0	-	-	0.0000	-	-
2016	Azores	8	0.0	0.0	0.0	0.0000	0.0000	0.0000
2017	Azores	4	6.3	10.5	5.3	0.0175	0.0315	0.0157
2018	Azores	9	1.8	1.9	0.6	0.0036	0.0061	0.0020
2019	Azores	38	2.6	3.3	0.5	0.0060	0.0101	0.0016
2020	Azores	9	1.9	2.2	0.7	0.0067	0.0111	0.0037
2021	Azores	23	1.0	1.6	0.3	0.0030	0.0062	0.0013
2022	Azores	9	3.1	6.8	2.3	0.0117	0.0319	0.0106
2016	Canaries	8	5.1	2.4	0.9	0.0002	0.0003	0.0002
2017	Canaries	2	6.0	8.5	6.0	0.0030	0.0042	0.0030

Table S2.3. Overall percentages of main plastic morphologies found in fledglings and adults of Cory's shearwater over the Azores and the Canaries.

Age class	Hard fragments	Threadlike items	Sheetlike items	Foam plastics	Plastic pellets	Other plastic
Fledglings	71.27%	20.89%	5.88%	0.27%	1.50%	0.18%
Adults	68.90%	14.90%	4.05%	6.76%	3.15%	2.25%



Figure S2.1. Examples of two Cory's shearwater gizzards found with several plastic items in the Azores archipelago.

Table S2.4. Summary information about the plastic sizes (mm) found in the stomachs of Cory's shearwater fledglings between breeding regions with size classes divided by large microplastics (1 - 5 mm), mesoplastics (5.1 – 25 mm) and macroplastics (>25 mm) for 2015 to 2017.

Information per plastic size class between breeding populations over the period 2015-2017						
Region	Size class (mm)	N° of plastics	%FO	Mean size (mm)	SD	SE
Azores	1 - 5	2230	92%	2.92	1.21	0.03
Azores	5.1 - 25	197	8%	7.2	2.36	0.17
Azores	>25	7	0.3%	45.14	17.58	6.65
Canaries	1 - 5	926	68%	2.17	1.18	0.04
Canaries	5.1 - 25	417	31%	9.97	4.6	0.23
Canaries	>25	24	2%	38.58	10.9	2.23

Table S2.5. Summary information about the plastic sizes (mm) found in the stomachs of Cory's shearwater fledglings in the Azores from 2015 to 2021.

Size information of plastics ingested by fledglings of Cory's shearwaters in the Azores archipelago				
Year	N° of plastics	Mean plastic sizes (mm)	SD	SE
2015	606	3.45	2.31	0.09
2016	777	3.40	2.49	0.09
2017	1054	3.34	3.61	0.11
2018	1281	4.81	10.40	0.29
2019	1021	4.31	6.30	0.20
2020	1569	4.29	5.00	0.13
2021	961	3.74	4.03	0.13

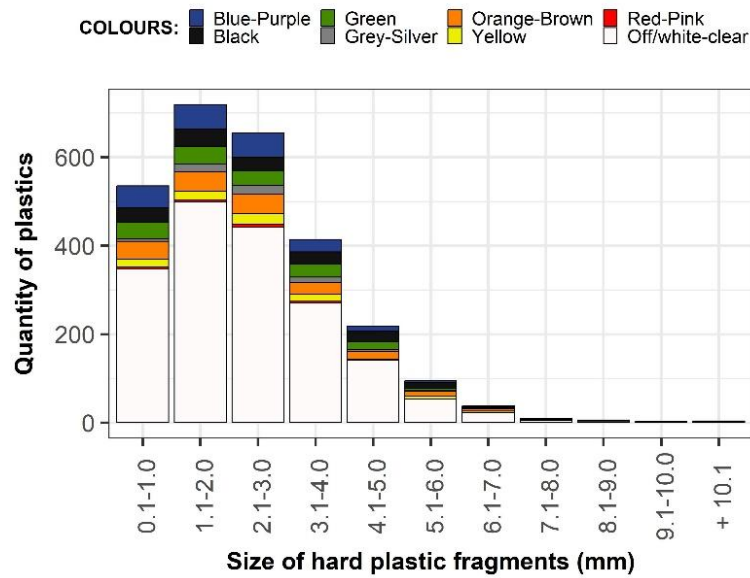


Figure S2.2. Frequency of sizes (mm) and colour composition of hard plastic fragments recovered from fledglings in the Azores and the Canaries between 2015 and 2017.

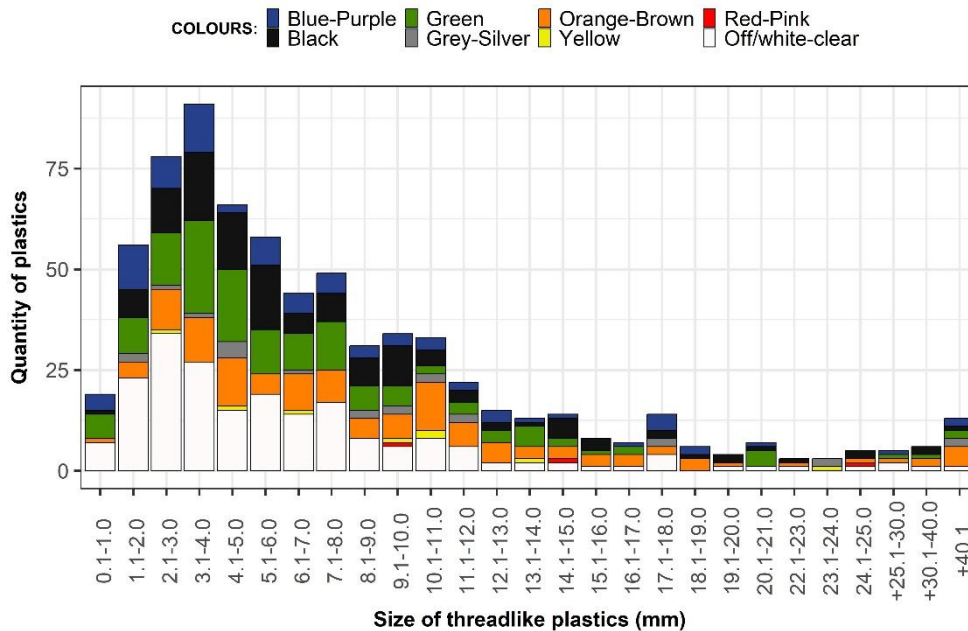


Figure S2.3. Frequency of sizes (mm) and colour composition of threadlike items recovered from fledglings in the Azores and the Canaries between 2015 and 2017.

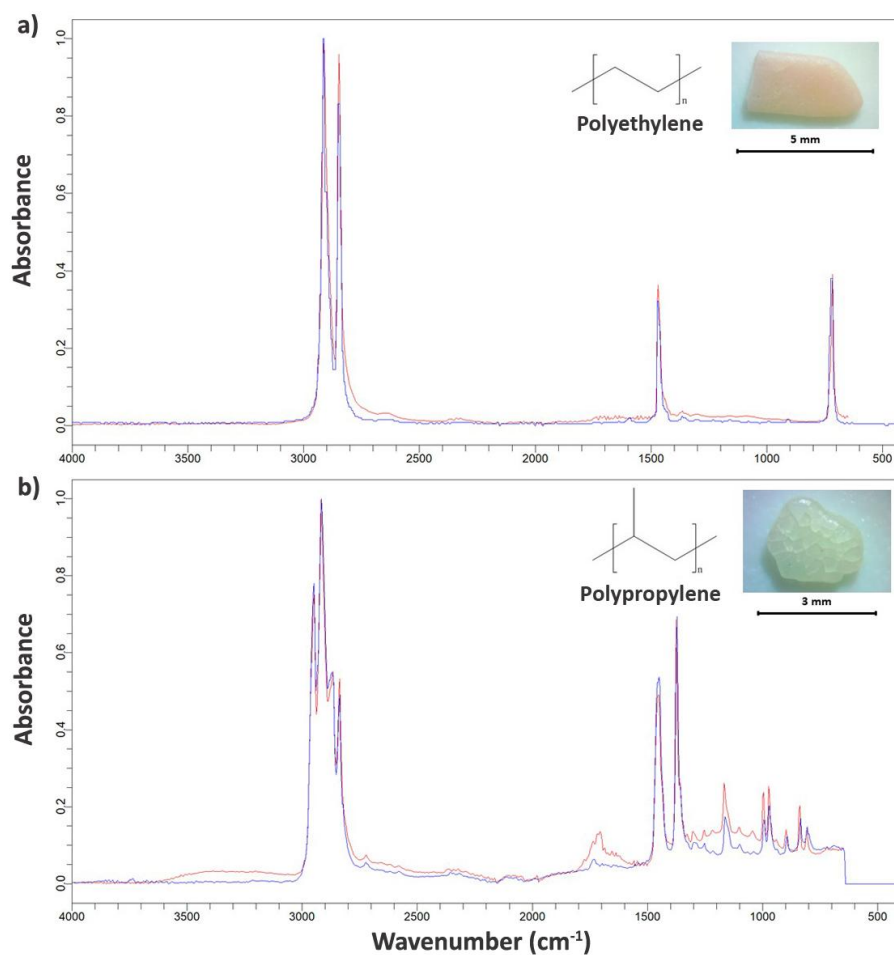


Figure S2.4. ATR-FTIR spectra of a) Polyethylene fragment (matching 96.4%) and b) Polypropylene fragment (matching 97.5%). The red line is the experimental spectra and the blue line is the library spectra.

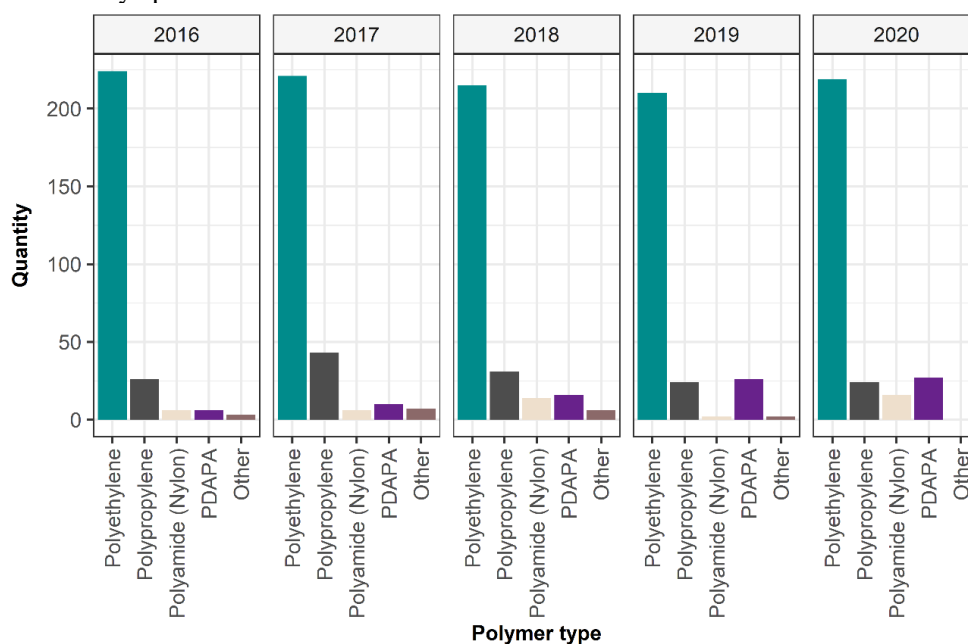


Figure S2.5. Polymer types obtained from the ATR-FTIR analysis of the plastics ingested by fledglings of Cory's shearwaters over five consecutive years (2016 to 2020). The PDAPA represents the poly(dimer acid-co-alkyl polyamine) and the category 'other' includes polyacrylate, styrene-ethylene/butylene-styrene (copolymer), polystyrene, an epoxy resin used as adhesive as well as polymers based on a mixture of polyamide and polyester.

Figure S2.6

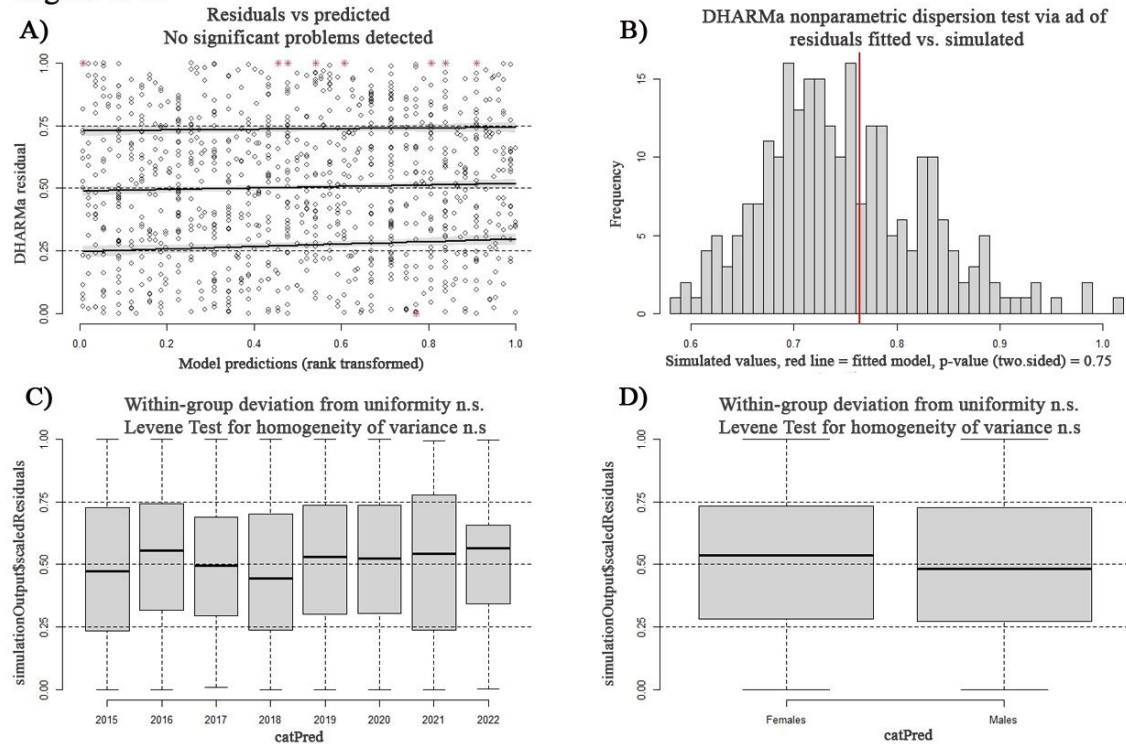


Figure S2.7

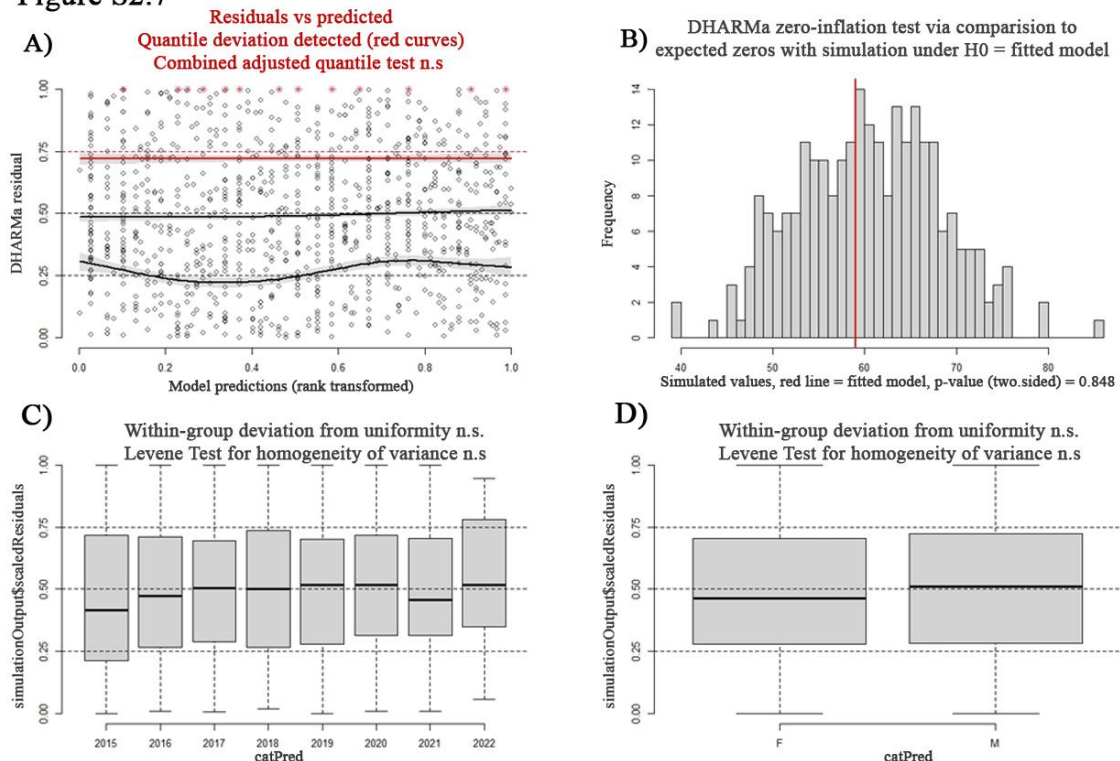


Figure S2.6 and Figure S2.7. Dharma validation plots of the models applied to investigate temporal trends in the number (S2.6) and mass (S2.7) of plastic ingested by Cory's shearwaters. Validation plots provide a good way to assess the performance and accuracy of the statistical approach since they help to understand how well the models fit the data and whether they capture the underlying patterns effectively. When validation plots show strong signals (signals are indicated in red with Dharma) some distributional assumptions are violated and thus, the models need to be readjusted, or in the worst cases, there is a model misfit.

A) Scaled quantile residuals versus (ranked) fitted values plots are used to check that the homogeneity and independence assumptions are reached. Plots A) from Fig.S2.6 and Fig.S2.7 have the residuals uniformly distributed meaning the models do not violate the assumptions, yet Fig. S2.7-A show a small quantile deviation. Since the lines do not show strong residual patterns, and the residuals seem to be randomly scattered everywhere, that small signal was not considered, and this validation plot was considered good. **B) Dispersion plots** are used to test over/underdispersion in which the histograms represent the data produced by the models whereas the red vertical line represents the original dataset. Plots B from Fig. S2.6 and Fig. S2.7 are good results showing that the variation on the original dataset (red line) is not small (under) or higher (over) dispersed than the information produced by the models, concluding that our models can cope with the variation in the original data. The **C) scaled quantile residuals versus year** and the **D) scaled quantile residuals versus sex** are plotted to check the homogeneity and independence of the variables included in the fixed part of the models (year and sex). The fact that all the boxplots are similar (in variation and distribution over the graphs) with any of them showing problems (which would be represented by boxplots in red with Dharma) means that we can trust the temporal results obtained and that the sex of fledglings does not have any effect on the ingestion of plastics.

Table S2.6. Summary outputs from the models applied to investigate temporal trends in the number of plastics (a) and the mass (b) ingested by Cory's shearwater fledglings.

a) Summary output number of plastics NB GAMM				
A. parametric coefficients	Estimate	Std. Error	t-value	p-value
(Intercept)	1.4063	0.0914	15.3829	< 0.0001
fYear2016	0.2153	0.1057	2.0372	0.0416
fYear2017	0.9609	0.113	8.505	< 0.0001
fYear2018	0.9286	0.1088	8.5377	< 0.0001
fYear2019	0.8891	0.1008	8.8215	< 0.0001
fYear2020	1.4301	0.0993	14.4045	< 0.0001
fYear2021	1.1111	0.1036	10.7211	< 0.0001
fYear2022	1.8747	0.133	14.0918	< 0.0001
SexMales	0.004	0.0512	0.0775	0.9382
B. smooth terms	edf	Ref.df	F-value	p-value
s(fIsland)	2.7194	7	5.3836	0.076
b) Summary output mass of plastics Tweedie GAMM				
A. parametric coefficients	Estimate	Std. Error	t-value	p-value
(Intercept)	-3.9937	0.0965	-41.4039	< 0.0001
fYear2016	0.0886	0.1257	0.7046	0.4812
fYear2017	0.3317	0.1369	2.4229	0.0156
fYear2018	-0.0356	0.1345	-0.2651	0.791
fYear2019	0.0822	0.1236	0.6648	0.5063
fYear2020	0.3022	0.1204	2.5101	0.0122
fYear2021	-0.3745	0.1319	-2.8385	0.0046
fYear2022	0.3261	0.1674	1.9474	0.0518
SexMales	-0.0915	0.0657	-1.3917	0.1643
B. smooth terms	edf	Ref.df	F-value	p-value
s(fIsland)	0.0232	7	0.003	0.4096

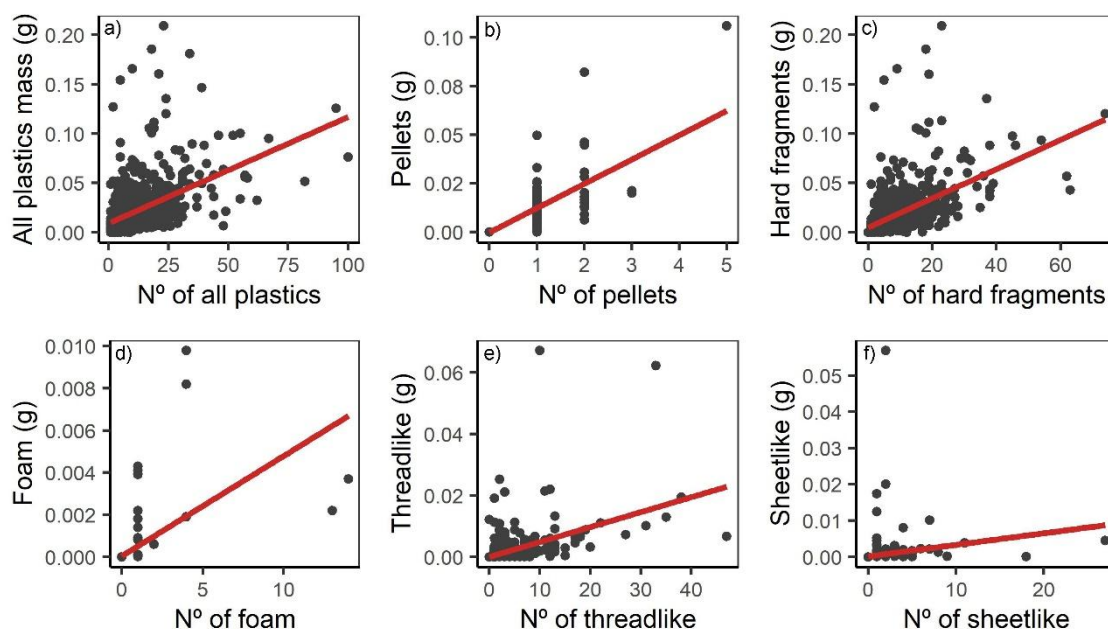


Figure S2.8. Relationship between the number of plastic items and their mass for a) all the morphologies together ($r = 0.5$), and individually ordered from higher to lowest correlations as follows: b) industrial plastic pellets ($r = 0.82$), c) hard plastic fragments ($r = 0.54$), d) foam plastics ($r = 0.57$), e) threadlike items ($r = 0.51$) and f) sheetlike items ($r = 0.21$).

Table S2.7. Percentiles of plastics ingested by Azorean fledglings ($n = 1,030$).

A) Number of plastics																
10%	15%	20%	25%	30%	35%	40%	45%	50%	55%	60%	65%	70%	75%	80%	85%	90%
1	2	3	4	4	5	6	7	8	9	10	11	12	14	16	20	23
B) Mass of plastics (g)																
10%	15%	20%	25%	30%	35%	40%	45%	50%	55%	60%	65%	70%	75%	80%	85%	90%
0.0002	0.0019	0.0030	0.0046	0.0061	0.0076	0.0093	0.0107	0.0126	0.0144	0.0169	0.0197	0.0221	0.0249	0.0287	0.0340	0.0426

Table S2.8. Number of plastics ingested between 2015 and 2022 by fledglings divided by the different islands of the Azores ordered from their location in the archipelago from west to east. The reporting metrics are the median, the arithmetic mean (mean), the standard deviation (SD), the standard error (SE), the residual standard deviation (RSD), and the residual standard error (RSE).

Islands ordered from W to E	Location in the region	N° fledglings	Median	Mean	SD	SE	RSD	RSE
Flores	Western	8	4.0	5.4	3.3	1.2	62	22
Corvo	Western	28	5.5	6.7	5.6	1.1	85	16
São Jorge	Central	133	5.0	8.5	9.6	0.8	113	10
Pico	Central	372	7.0	10.4	13.6	0.7	131	7
Faial	Central	381	9.0	11.6	11.9	0.6	102	5
Graciosa	Central	17	13.0	13.5	7.8	1.9	57	14
São Miguel	Eastern	62	10.5	13.2	11.1	1.4	84	11
Santa Maria	Eastern	27	15.0	15.4	11.5	2.2	75	14

Table S2.9. Plastic ingested from 2015 to 2022 by A) fledglings Cory's shearwaters victims of light pollution found dead during the fledging season in the Azores archipelago and B) northern fulmars found dead on beaches in the Netherlands (including adults and non-adults), visualized over periods of five years. All plastic categories are combined in these tables, including loads from the entire stomach, including the proventriculus and gizzard. The general information reported for each period includes the sample size (N), the percentage of adults in the sample (% adults), the percentage frequency of occurrence of plastic ingestion (%FO), and the mean number and mass with the standard errors (SE). In addition, the TV% is included in the last column of both tables representing in Table A) the percentage of fledglings having more than the limit value of 4 plastic particles selected for Cory's shearwaters (Cory's-TV%) and in Table B) the number of northern fulmars having more than 0.1g of plastics (Fulmar-TV%). Information from Table B was copied from Table A of the latest fulmar litter monitoring report (Kühn et al., 2023).

A) Total plastics found on fledglings Cory's shearwaters in the Azores (this study)						
Periods	N	% adults	%FO	Mean number ± SE	Mean mass (g) ± SE	Cory's-TV% (>4 plastics) *
2015-2019	675	0%	92%	8.0 ± 0.4	0.0195 ± 0.0011	60%
2016-2020	689	0%	95%	11.0 ± 0.5	0.0208 ± 0.0011	73%
2017-2021	671	0%	95%	12.6 ± 0.5	0.0194 ± 0.0010	80%
2018-2022	626	0%	95%	13.9 ± 0.6	0.0191 ± 0.0010	81%

B) Total plastics found in northern fulmars in the Netherlands (Kühn et al., 2023)						
Periods	N	% adults	%FO	Mean number ± SE	Mean mass (g) ± SE	Fulmar-TV% (over 0.1g) *
2015-2019	148	30%	93%	20.1 ± 4.6	0.20 ± 0.04	39%
2016-2020	139	28%	93%	22.5 ± 5.2	0.21 ± 0.04	39%
2017-2021	184	27%	95%	26.0 ± 4.4	0.24 ± 0.04	48%
2018-2022	212	30%	95%	23.5 ± 3.2	0.26 ± 0.04	50%

* The TV% (percentage of birds over the limit value of plastics established) for Cory's shearwaters (A) and northern fulmars (B) can be compared directly by correcting them for the allowed exceedance percentages established for each (Cory's-TV% - 20% and Fulmar-TV% - 10%). The overall Cory's-TV is the combination of 4 plastic items and the maximum exceedance of 20%.

References

Kühn S., Meijboom A., Bittner O., & Van Franeker J.A. 2023. Fulmar Litter Monitoring in the Netherlands – Update 2022. Wageningen Marine Research Report C039/23 and RWS Centrale Informatievoorziening BM 23.14; pp 51 doi: <https://doi.org/10.18174/633324>

Appendix B – Supplementary Material chapter III

Source: Rodríguez, Y., Rodríguez, A., Pereira, J.M. and Pham, C.K., 2025. Plastics reset in an adult Procellariiform seabird species during the breeding season. *Marine Environmental Research*, p.106939. <https://doi.org/10.1016/j.marenvres.2024.106939>



Figure S3.1. Map of the Atlantic Ocean identifying the breeding region where the samples of dead adults Cory's shearwaters (*Calonectris borealis*) were collected for the study. The annual distribution of the studied species was taken from BirdLife International (Downloaded on 20/08/2024 from <https://datazone.birdlife.org/species/factsheet/corys-shearwater-calonectris-borealis>). The main breeding and foraging area where the birds were studied was approximately identified.

Table S3.1. Percentage frequency of occurrence (%FO) of plastic ingestion during the nesting periods and the two stomach sections that compose the stomachs, the proventriculus, and the gizzard.

	Proventriculus %FO	Gizzard %FO	z-value	p-value	Overall %FO
Pre-laying	18%	65%	3.9	<0.0001	68%
Incubation	30%	60%	1.9	0.05	75%
Chick-rearing	10%	29%	4.4	<0.0001	36%

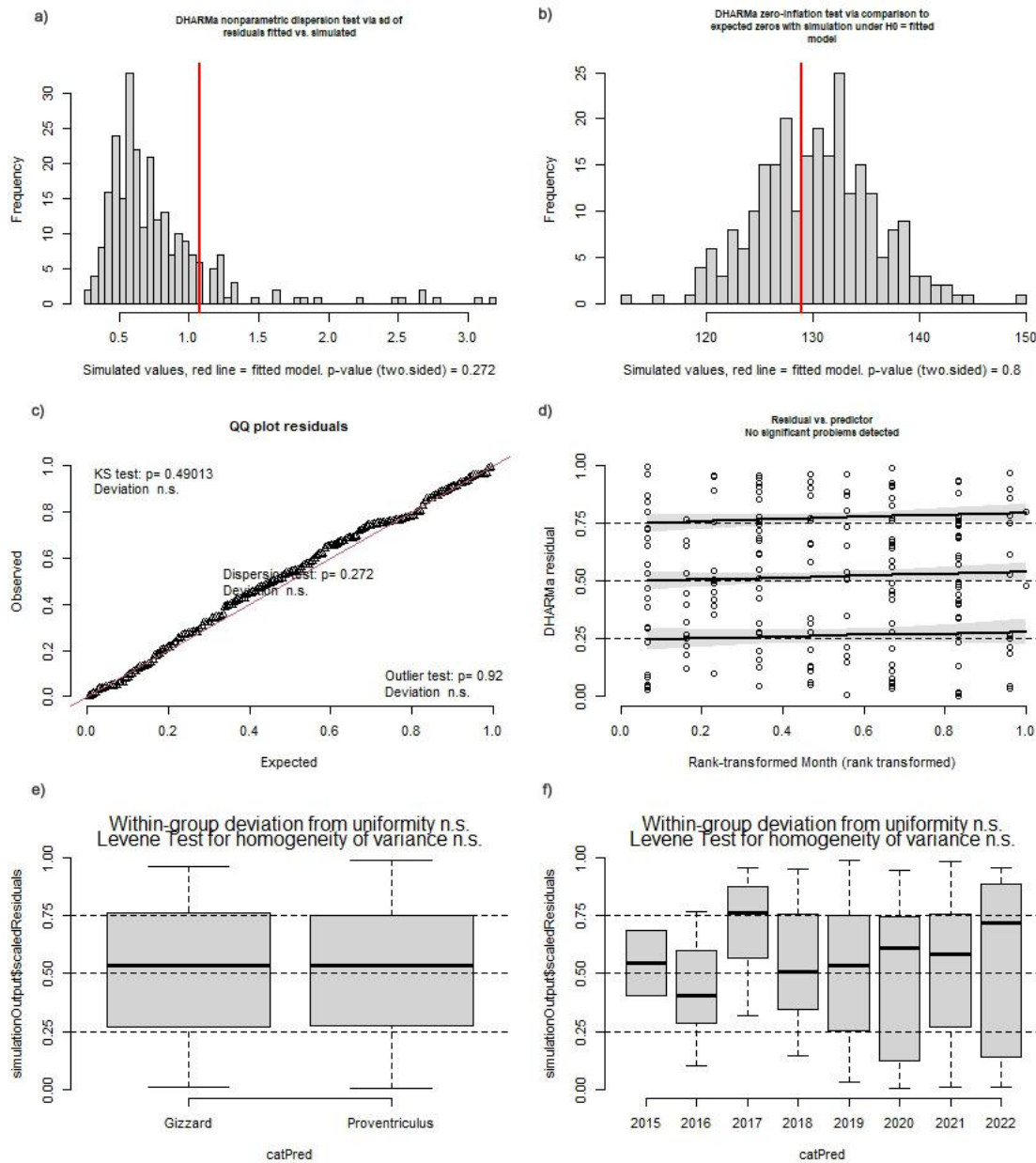


Figure S3.2. DHARMa validation plots showing a good performance of the applied GAMM model to investigate temporal trends in plastic loads over a breeding season in adults Cory's shearwaters. a) Simulation study (represented by the grey bars) to test the dispersion statistic not showing either overdispersion or underdispersion, which is indicated by the position of the red vertical line (dispersion of the original dataset) between the simulated data. b) Simulation study (represented by the grey bars) to test zero inflation, showing that the model can cope with the numbers of zeros in the original dataset (indicated by the red vertical line). c) QQ plot to verify the normality, showing that quantile residuals are uniformly distributed. d) Residuals versus predictors to check the independence of the variable 'month', showing no patterns. e) Levene test to check the homogeneity of the variable 'stomach section' included in the model, showing no patterns in the residuals. f) Levene test to check the homogeneity of the variable 'year' (not included in the model), showing no patterns in the residuals. This indicates a non-temporal correlation with 'year' and no concern for the absence of this variable in the model ensuring the reliability of the results.

Table S3.2. Dunn test outputs obtained after the Kruskal-Wallis were statistically significant. These tests were applied to investigate differences in A) the number of plastics and B) the mass of plastics found in the stomachs of adult Cory's shearwaters among the three breeding periods. The periods were defined by temporal order as pre-laying, incubation, and chick rearing. The comparison matrix contains the z-values (above) and adjusted p-values (bottom) for each pairwise comparison. The statistical significance was set at a p-value < 0.05 indicated with an * in the table.

Col Mean-Row mean	A) Comparison of x by group (Bonferroni)	
	Incubation period	Chick-rearing period
Pre-laying period	-0.050743 1	-3.851438 0.0002*
Incubation period		-3.217830 0.0019*

Col Mean-Row mean	B) Comparison of x by group (Bonferroni)	
	Incubation period	Chick-rearing period
Pre-laying period	0.016501 1	-3.711986 0.0003*
Incubation period		-3.169164 0.0023*

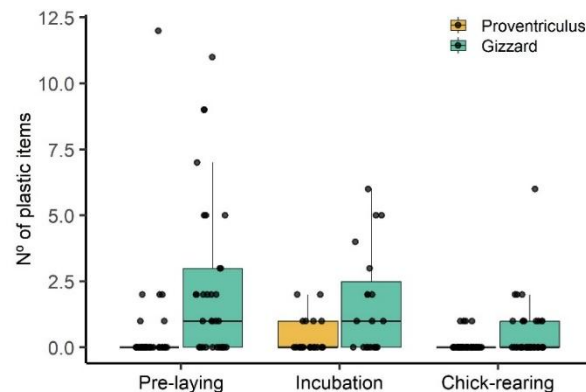


Figure S3.3. Boxplots showing the number of plastics found among the three breeding periods and the two stomach sections that compose the stomach. The middle value of the dataset (50th percentile or median value) is shown as the median line inside the box. A jittering was added to visualize the original data (black dots).

Table S3.3. Dunn test output obtained after a statistically significant result from a Kruskal-Wallis test to investigate differences in the plastic size (mm) among the two stomach sections that compose the stomachs of adult Cory's shearwaters among three breeding periods. The comparison matrix contains the z-values and adjusted p-values for each pairwise comparison. The statistical significance was set at a p-value < 0.05 indicated with an * in the table.

Col Mean-Row mean	Comparison of x by group (Bonferroni)				
	Gizzards chick rearing	Proventriculus chick rearing	Gizzards incubation	Proventriculus incubation	Gizzard pre-laying
Proventriculus chick rearing	-0.533730 1.0000				
Gizzard incubation	0.766942 1.0000	0.989085 1.0000			
Proventriculus incubation	1.618421 0.7918	1.638987 0.7591	1.147576 1.0000		
Gizzard pre-laying	0.072780 1.0000	0.615057 1.0000	-0.969067 1.0000	-1.770256 0.5751	
Proventriculus pre-laying	1.190975 1.0000	1.245102 1.0000	0.488178 1.0000	-0.807758 1.0000	1.502276 0.9977

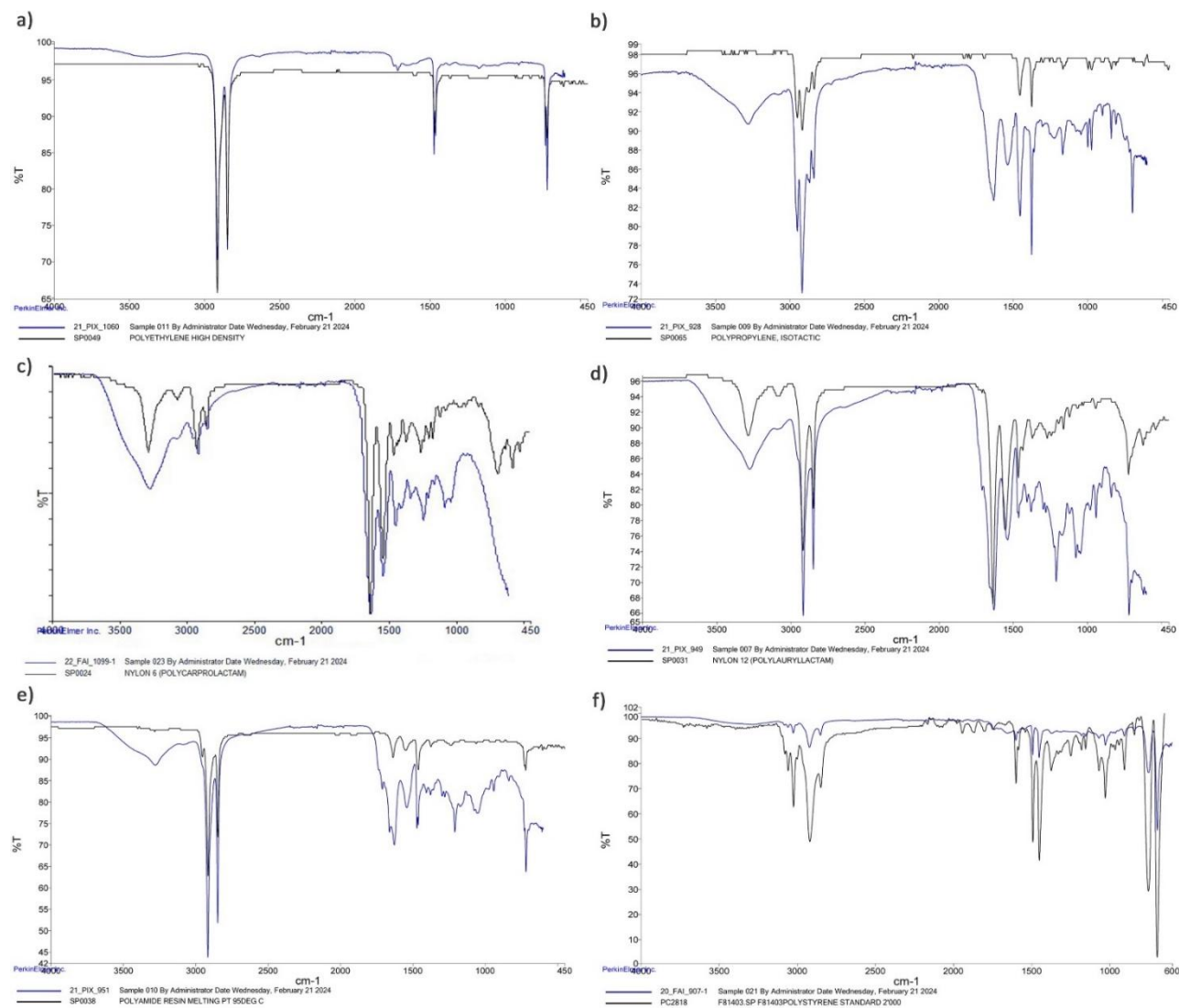


Figure S3.4. FTIR results showing overlapped both, the library and the plastic sample spectra, for the following polymer types ingested by Cory’s shearwaters: a) polyethylene high density, b) polypropylene, c) nylon 6, d) nylon 12, e) polyamide resin and f) polystyrene. The commercial libraries used were provided by PerkinElmer and are as follows: “adhes”, “polyatr”, “ATRSPE~1”, “FIBERS3”, “InPoly.sp1”, “POLY1”, “POLYADD1”, “POLYMER”, “SOLVENT1”. The %T in the y-axis means percentage of transmittance.

Appendix C – Supplementary Material chapter IV

Source: Rodríguez, Y., Silva, M.A., Pham, C.K. and Duncan, E.M., 2023. Cetaceans playing with single-use plastics (SUPs): A widespread interaction with likely severe impacts. *Marine Pollution Bulletin*, 194, p.115428. <https://doi.org/10.1016/j.marpolbul.2023.115428>

Table S4.1. Records of cetaceans interacting with plastic litter on social media websites. The data identified with * represent those events classified as play with uncertainty due to the reduced available visual material to be analysed.

Common name	Location	Ocean basin	Year of the publication
<i>Physeter macrocephalus</i>	Azores - Portugal	Atlantic	2008
<i>Stenella longirostris</i> *	Hawaii - USA	Pacific	2009
<i>Tursiops truncatus</i> *	Canaries - Spain	Atlantic	2011
<i>Stenella longirostris</i> *	Fernando Noronha - Brazil	Atlantic	2011
<i>Orcinus orca</i>	Salish Sea - USA	Pacific	2013
<i>Sotalia guianensis</i> *	Baía de Guanabara - Brazil	Atlantic	2015
<i>Tursiops truncatus</i>	Madeira - Portugal	Atlantic	2015
<i>Tursiops truncatus</i>	Egypt	Red Sea	2015
<i>Stenella longirostris</i> *	Hawaii - USA	Pacific	2016
<i>Stenella longirostris</i> *	Hawaii - USA	Pacific	2016
<i>Tursiops truncatus</i> *	Unknown	Pacific	2016
<i>Tursiops truncatus</i> *	Canaries - Spain	Atlantic	2016
<i>Tursiops truncatus</i> *	Canaries - Spain	Atlantic	2017
<i>Stenella longirostris</i> *	Hawaii - USA	Pacific	2018
<i>Tursiops truncatus</i> *	Unknown	Unknown	2018
<i>Physeter macrocephalus</i> *	Azores - Portugal	Atlantic	2018
<i>Tursiops truncatus</i>	Grand Turk Island - UK	Atlantic	2018
<i>Stenella longirostris</i>	Unknown	Unknown	2019
<i>Tursiops truncatus</i>	Egypt	Red Sea	2019
<i>Stenella longirostris</i>	Unknown	Unknown	2019
<i>Stenella longirostris</i>	Hawaii - USA	Pacific	2019
<i>Stenella longirostris</i>	Egypt	Red Sea	2019
<i>Stenella longirostris</i>	Egypt	Red Sea	2019
<i>Tursiops truncatus</i> *	Egypt	Red Sea	2020
<i>Delphinus delphis</i>	Gibraltar - UK	Mediterranean Sea	2020
<i>Tursiops truncatus</i>	Hawaii - USA	Pacific	2020
<i>Stenella longirostris</i> *	Bonin Islands - Japan	Pacific	2021
<i>Stenella longirostris</i>	Hawaii - USA	Pacific	2021
<i>Stenella frontalis</i>	Bahamas	Atlantic	2021
<i>Tursiops truncatus</i>	Canaries - Spain	Atlantic	2022
<i>Tursiops truncatus</i> *	Canaries - Spain	Atlantic	2022
<i>Physeter macrocephalus</i>	Mauritius	Indian	2022
<i>Pseudorca crassidens</i>	Azores - Portugal	Atlantic	2022
<i>Tursiops truncatus</i> *	Bahamas	Atlantic	2022

Table S4.2. Details of the play events recorded between cetaceans and plastic litter in the Azores (NE Atlantic) by a tour operator over 12 years.

Species	Year	Plastic shape	Plastic colour	Plastic location on the body
<i>Delphinus delphis</i>	2011	Plastic bag	White	Head
<i>Tursiops truncatus</i>	2011	Plastic bag	Yellow	Dorsal fin
<i>Tursiops truncatus</i>	2013	Sheetlike	Yellow	Dorsal fin
<i>Tursiops truncatus</i>	2013	Sheetlike	Red	Head
<i>Tursiops truncatus</i>	2014	Plastic bag	White	Dorsal fin
<i>Tursiops truncatus</i>	2014	Sheetlike	Yellow	Dorsal fin
<i>Grampus griseus</i>	2016	Plastic bag	Blue	Tail
<i>Grampus griseus</i>	2017	Sheetlike	White	Dorsal fin
<i>Grampus griseus</i>	2017	Sheetlike	White	Dorsal fin
<i>Grampus griseus</i>	2017	Sheetlike	Yellow	Dorsal fin
<i>Tursiops truncatus</i>	2018	Plastic bag	White	Head
<i>Tursiops truncatus</i>	2018	Sheetlike	Yellow	Tail
<i>Tursiops truncatus</i>	2018	Sheetlike	Transparent	Dorsal fin
<i>Tursiops truncatus</i>	2018	Plastic bag	White	Dorsal fin
<i>Tursiops truncatus</i>	2018	Plastic bag	Blue	Tail
<i>Physeter macrocephalus</i>	2019	Raffia sack	White	Head
<i>Tursiops truncatus</i>	2019	Sheetlike	Blue	Head
<i>Tursiops truncatus</i>	2020	Sheetlike	Transparent	Dorsal fin
<i>Physeter macrocephalus</i>	2020	Hard plastic box	Black	Head
<i>Tursiops truncatus</i>	2022	Plastic bag	Black	Dorsal fin
<i>Physeter macrocephalus</i>	2022	Hard plastic box	Black	Head

Appendix D – Supplementary Material chapter V

Source: Rodríguez, Y., Vandeperre, F., Duncan, E.M., Machete, M. and Pham, C.K., 2025. An index to differentiate megafauna entangled in operational fishing gears from abandoned, lost, or otherwise discarded fishing gears. *Science of The Total Environment*, 971, p.178950. <https://doi.org/10.1016/j.scitotenv.2025.178950>

Table S5.1. Detailed criteria description for the proper application of the ENSA index. This index only applies to events of marine megafauna found entangled in fishing-related items. Therefore, it cannot be used in entanglement events involving user plastics (e.g., plastic bags, plastic rings, or any other type of single-use plastic).

Integrity	This ENSA criterion based on the principle that items used in operational fisheries, and/or fishing gears that are found complete, are more likely to suggest an entanglement in OFG. This would be the case of an animal found entangled in part of a fishing line if it has a hook in its mouth or an animal entangled in fishing gear such as a complete gillnet. In contrast, an animal entangled in a single accessory (e.g., ropes), or in a portion of a gear (e.g., a net cutting, fishing lines without hooks) is more likely to indicate an entanglement in ALDFG, as small gear part are often discarded into the environment intentionally or accidentally (e.g., Metcalfe & Bentley, 2020). In the cases of megafauna entangled in static fishing gear, the ‘integrity’ criterion will not point to ALDFG. Although ropes are considered fishing accessories, these animals are typically found entangled in the complete (scoring towards zero), or almost complete (scoring towards 0.5) gear setup (e.g., rope, buoys, and pots). Therefore, in such a case, this criterion will indicate a high probability of an entanglement in OFG.
Composition	Refers to the number of items the animal is entangled in. It is designed to indicate a higher probability of an OFG when the item can be directly identified as a single gear type, even if it is not complete (e.g., only a section of a net), or when it consists of more than one element if they are related to the original gear (e.g., a rope with a buoy and a pot). On the contrary, an entangling item that is a conglomerate (defined as a group of entangling items noticeably unrelated to a single fishery) implies that those items have been part of marine litter for a while. Hence, scoring towards one or a high likelihood of entanglement in ALDFG. Conglomerates might also contain items not associated with any fishing activity like user plastics (e.g., bags, boxes, fragments), which further indicates the item to be ALDFG.
Origin	This is the criterion that requires the most expert knowledge. A fishing item commonly used in the area where the incident is recorded will lead to the classification of the event as more likely caused by an OFG. On the contrary, an organism entangled in a fishing item not used locally is more likely to point to an entanglement in ALDFG. It is possible that an entangled animal in OFG in a distant region got lost and drifted to another area where it was then eventually found. In such a hypothetical case, because the gear is not local, the criterion ‘origin’ will misclassify that event as caused by ALDFG. Yet, this criterion will be counterbalanced by the others since the gear might be complete (‘integrity’ scoring zero), might has no other types of gears or litter associated (‘composition’ scoring zero), might be well preserved (‘condition’ scoring zero) and might does not have biota growing on it (‘biofouling’ scoring zero). In such cases, the overall scoring of the index will correctly classify such entanglement as caused by an interaction with an OFG even if the criterion ‘origin’ classified as ALDFG.

Condition	<p>A criterion related to the gear status and its weathering. A new or well-preserved fishing item suggests an entanglement in an OFG. On the contrary, a weathered/damaged fishing item typically indicates an object floating as an ALDFG for a certain period of time.</p> <p>Importantly, since most fishing items are commonly made of plastic, and plastic is known for its durability, certain polymers are very resistant to degradation in the marine environment even after drifting for a prolonged period (e.g., longlines). Yet, with time, they will eventually become highly entangled in themselves. As a result, a fishing item that appears new, but in a state of disarray, will also indicate that it has been drifting for some time as an ALDFG.</p>
Biofouling	<p>This criterion was defined so that, if a fishing item does not have organisms growing over it, it will suggest that it has been in recent operation. Consequently, pushing the scoring toward zero. While it is true that OFG can also hold some organisms on their surface, it is generally the non-operational gears that tend to accumulate more biofouling. Hence, a fishing item covered with visible biofouling (e.g., barnacles) will more likely point to an item that has drifted passively as an ALDFG.</p> <p>Note that the ‘biofouling’ criterion is not correlated to the ‘condition’ criterion as it is possible to find an old or damaged fishing item without biofouling, or a seemingly new ALDFG with biofouling. In addition, certain floating object that are also known to cause entanglements (Fisheries NOAA, 2023), such as static fish aggregating devices also known as FADs, can also be found with biofouling when active (Shuto & Hayashi, 2013).</p>
Status	<p>This is the only criterion that directly assesses the external health of the entangled animal. It was established based on the premise that entanglements resulting from OFG would more likely cause immediate harm or death, particularly when animals become entangled in an entire OFG. Common reasons for animals being found subsequently adrift after being entangled by an OFG include those that dragged or broke fishing gears, fishers that release entangled animals along with part of the gear, or gears that became lost due to bad weather. In all those cases, even if the initial interaction was not directly fatal, the animal entangled in an OFG must first be lost before it can be found. It is therefore rather unlikely to be found in a healthy condition. On the contrary, a healthy animal points to an event that occurred more recently and is thus more likely an ALDFG entanglement. Animals with long-term injuries (e.g., necrosis or malformations) are included in the classification related to ALDFG since it is assumed that these injuries result from animals being trapped in items that, while not immediately fatal, severely impair their functionality. This is often the case with smaller items commonly associated with marine litter (e.g., ropes). However, in certain entanglements with OFG, some taxa such as turtles and whales might be able to carry fishing gear around their flippers for months. In those latter cases, even if the ‘status’ criterion classifies the event incorrectly, the other criteria will balance the final ENSA scoring towards an OFG or to an ambiguous event.</p>

References

- Fisheries, N.O.A.A. 2023. Fishing gear: fish aggregating devices <https://www.fisheries.noaa.gov/national/bycatch/fishing-gear-fish-aggregating-devices>.
- Metcalfe, R. and Bentley, A., 2020. Best Practices to reduce marine litter from net cuttings waste.
- Shuto, T. and Hayashi, R., 2013. Floating castles in the ocean: the barnacles of two giant fish aggregating devices from Okinawa, Japan. *Landscape and ecological engineering*, 9, pp.157-163. <https://doi.org/10.1007/s11355-012-0190-7>

Table S5.2. Detailed scores with associated weights per criterion and final ENSA value for each event. Some events were included as a control (*) and some are exemplified with pictures in Figures 2 and 3 (#).

ID	Taxa	Integrity (25%)	Composition (12%)	Origin (20%)	Condition (20%)	Biofouling (5%)	Status (18%)	ENSA index final score
Ent01	Sea turtle	25	12	20	20	0	18	95
Ent02*	Shark	0	0	0	0	0	18	18
Ent03	Sea turtle	25	0	20	10	2.5	18	75.5
Ent04	Sea turtle	25	12	20	10	0	0	67
Ent05	Sea turtle	25	12	10	20	0	18	85
Ent06#	Cetacean	25	0	20	10	2.5	18	75.5
Ent07	Sea turtle	25	12	20	0	0	18	75
Ent08	Cetacean	0	0	10	0	0	18	28
Ent09	Sea turtle	12.5	0	0	20	0	18	50.5
Ent10	Sea turtle	12.5	0	0	20	0	18	50.5
Ent11*	Sea turtle	0	0	0	0	0	9	9
Ent12*	Cetacean	0	0	0	0	0	18	18
Ent13	Sea turtle	25	0	20	0	0	18	63
Ent14	Sea turtle	25	0	10	20	5	18	78
Ent15*	Cetacean	0	0	0	0	0	18	18
Ent16	Sea turtle	25	0	20	0	0	18	63
Ent17	Sea turtle	25	0	20	20	0	9	74
Ent18	Sea turtle	25	12	20	20	2.5	18	97.5
Ent19	Seabird	25	12	20	20	2.5	9	88.5
Ent20	Sea turtle	25	0	20	0	0	18	63
Ent21	Cetacean	25	0	20	0	0	18	63
Ent22#	Sea turtle	25	12	20	20	5	18	100
Ent23#	Sea turtle	25	0	20	20	5	18	88
Ent24#	Cetacean	0	0	0	0	0	0	0
Ent25	Shark	25	0	20	0	0	18	63
Ent26	Shark	25	0	10	0	5	18	58
Ent27	Shark	25	0	10	10	5	18	68
Ent28*#	SeaTurtle	0	0	0	0	0	0	0
Ent29	Seabird	25	0	20	0	0	9	54
Ent30	Cetacean	25	0	20	0	0	18	63
Ent31	Cetacean	0	0	0	0	0	18	18
Ent32	Sea turtle	12.5	0	20	20	2.5	0	55
Ent33#	Sea turtle	12.5	0	20	20	2.5	0	55
Ent34	Shark	25	0	10	10	5	18	68
Ent35	Cetacean	25	0	10	0	0	18	53

Annex I - OSPAR’s Coordinated Environmental Monitoring Programme (CEMP) guidelines for a Common Indicator



**OSPAR
COMMISSION**

*Protecting and conserving the
North-East Atlantic and its resources*

CEMP Guidelines for monitoring and assessment of plastic litter ingested by fledglings of Cory’s shearwaters (*Calonectris borealis*) in OSPAR Region V

(OSPAR Agreement 2025-16)¹ OSPAR

Theme: Human activities

OSPAR descriptor: Marine litter

Indicator full name: abundance, composition, and trends of marine litter ingested by fledgling of Cory’s shearwaters

MSFD correspondence²:

Descriptor: 10 - Marine Litter

Criterion: D10C3 - The amount of litter and micro-litter ingested by marine animals is at a level that does not adversely affect the health of the species concerned.

Indicator: D10C3 – Amount of litter/micro-litter in grams (g) and number of items per individual for each species in relation to size (weight or length, as appropriate) of the individual sampled

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Supporting authors: Pedro Sepúlveda and Willem van Loon

Date: 09 - April 2025

Link to the official document with annexes: <https://www.ospar.org/documents?v=63717>

¹ English only

² Commission Decision (EU) 2017/848 of 17 May 2017, laying down criteria and methodological standards on good environmental status of marine waters and specifications and standardised methods for monitoring and assessment, and repealing Decision 2010/477/EU

1. Introduction

Cory’s shearwater (*Calonectris borealis*; Fig. 1) is a Procellariiform seabird with a widespread distribution across the Atlantic basin (BirdLife International, 2023). Its pelagic lifestyle keeps this species largely distant from terrestrial areas, except during the breeding season when large colonies congregate mostly in the Northeast (NE) Atlantic where they have been extensively studied (e.g., González-Solís et al., 2007; Magalhães et al., 2008; Paiva et al., 2010; Neves et al., 2012; Ramos et al., 2013; Atchoi et al., 2021). Their breeding cycle begins every year between February and March, as adults return to breeding sites across various oceanic archipelagos. Major colonies are established in the Azores archipelago, Madeira (Selvagens Islands), and the Canary Islands, while smaller colonies are located in mainland areas of Portugal, Spain, and the Alboran Sea (BirdLife International, 2018). During the post-breeding season, adults typically migrate to biomes associated with prominent upwelling in the South Atlantic, such as the Benguela and Agulhas Currents, as well as the southern segment of the Brazil Current (BirdLife International, 2023). Yet, some adults remain in the North Atlantic throughout the winter.

Portugal, through the Regional Government of the Azores (hereafter RGA), has proposed using Cory’s shearwater fledglings as an indicator for monitoring plastic pollution in the Azores archipelago. Initially, data from plastic ingestion in Cory’s shearwaters was included in the reporting to the Marine Strategy Framework Directive hereafter MSFD (EC, 2008) in 2020. Specifically, the species was used for the Descriptor 10, Criterion 3 (D10C3) “the amount of litter and micro-litter ingested by marine animals is at a level that does not adversely affect the health of the species”, although the impact of ingested plastics has not yet been evaluated. Subsequently, the potential use of this species as a bioindicator within OSPAR region V was presented to the Intersessional Correspondence Group on Marine Litter (ICG-ML). However, the formal implementation of a long-term monitoring program, managed by the Azorean administration, was delayed pending the completion of data collection from ongoing scientific studies. Recently, an initial eight-year assessment (2015-2022) confirmed that Cory’s shearwater fledglings display several promising attributes that make them a bioindicator of environmental plastic contamination (Rodríguez et al., 2024). In 2020, during the 2nd assessment of the MSFD for the Azores (MM, SRMCT, SRAAC, 2020), the RGA designated Cory’s shearwater as a bioindicator with a target (D10-AZO-M1) to be achieved by 2024. This will be implemented in the 3rd assessment report to be submitted to the MSFD in 2025.

Implementing Cory’s shearwater as a bioindicator will benefit OSPAR Region V, which currently does not have an effective species to monitor plastic particles in the size range of 1 to 25 mm. This region lies outside the distribution range of the northern fulmar (*Fulmarus glacialis*), a seabird species that has long been used as a bioindicator within the OSPAR and MSFD frameworks by various countries in the North Sea, Arctic waters, and Celtic Seas (OSPAR, 2015; EC, 2017). Other taxa, such as sea turtles, have recently been proposed as OSPAR bioindicators for different regions, including the EU-Macaronesia (Darmon et al., 2022; Galgani et al., 2022). Yet, collecting a significant quantity of samples in remote and small oceanic archipelagos like the Azores remains challenging. Moreover, the turtle carcasses in this region usually vary in age, size, body condition, and cause of death (Pham et al., 2017; Rodríguez et al., 2022), which makes the assessment of plastic contamination less reliable when using this species. The plastic size range and the plastic morphologies ingested by sea turtles (normally >25mm) also differ from those ingested by northern fulmars, making the plastic target attained by both bioindicators

different. Therefore, implementing a Cory’s shearwater monitoring program would benefit OSPAR and the MSFD by covering regions beyond the distribution range of northern fulmars in the North Atlantic, while addressing similar morphologies and plastic sizes among both seabird species. In addition to marine litter, there is a political need to monitor other anthropogenic threats. In this sense, Cory’s shearwaters have already been suggested as possible biomonitors of heavy metals across the Atlantic basin, with particular focus on mercury (Gatt et al., 2020). As an indicator for heavy metals, this species would also contribute to assessing Descriptor 8 under the MSFD (Criterion D8C1). Thus, the species shows potential as a bioindicator for multiple threats, provided that collaborative efforts are aligned among institutions.

Overall, this document outlines guidelines for a formal Cory’s shearwater monitoring program within the OSPAR framework to assess floating plastic contamination in OSPAR Region V. This document covers the collection of dead birds, the target sample age, how to perform the sampling systematically, the storage and analysis of the gathered data, and projected goals.



Figure 1. Picture taken during the release of a rescued Cory’s shearwater fledgling during a rescue campaign in the Azores archipelago. (Photo credit: Catarina Fazenda, Observatório do Mar dos Açores – OMA).

2. Monitoring

2.1. Purpose

The primary aim of this monitoring program is to provide data to address the abundance, composition, and trends of floating plastics in offshore waters of the Northeast Atlantic, using Cory’s shearwaters fledglings as indicators. This indicator will focus on all visible items with a focus on smaller sizes since it has been demonstrated that Cory’s shearwaters mainly ingest microplastics (1-5 mm) and mesoplastics (5-25 mm) (Rodríguez et al., 2012; Rodríguez et al., 2024). Long-term monitoring of fledglings would yield valuable insights into plastic pollution dynamics and sources, allowing for accurate tracking of plastic litter over time (Rodríguez et al., 2024).

2.2. Quantitative Objectives

Our proposal for their applicability within an environmental monitoring framework would be to achieve only 20% of Cory’s shearwaters fledglings (collected dead only during fledgling seasons) with more than 4 plastic particles in their stomachs in a sample of a minimum of 40 birds per year and assessment area/subregion. Cory’s shearwaters breeding areas span multiple regions in the Northeast Atlantic Ocean (Fig. 2), with one of the most important colonies being based in the Azores archipelago (OSPAR Area V). Additionally, Madeira (Portugal) and the Canary Islands (Spain) host other significant breeding colonies of Cory’s shearwaters, although these regions are not currently part of the OSPAR area.

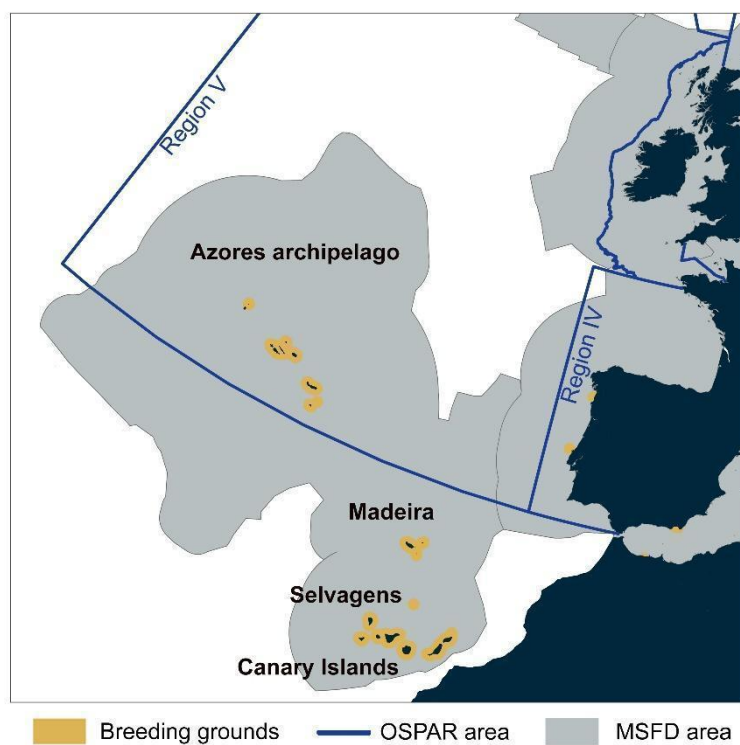


Figure 2. Location of Cory’s shearwater breeding grounds in the North-East Atlantic and the Western Mediterranean Sea (Source: BirdLife International) identifying the overlapping OSPAR and MSFD policy regions.

2.3. Monitoring Strategy

2.3.1. Target age

This monitoring proposal is intended to be based exclusively on fledglings collected dead during the fledging season. These fledglings have not been fed independently, as they died before reaching the ocean. Therefore, their stomach contents represent the plastics provided solely by both parents during the chick-rearing period (Rodríguez et al., 2024; Rodríguez et al., 2025). While fledging, light pollution makes otherwise healthy fledglings vulnerable to collisions, road accidents, or predation. Since the fledging season covers 30 days (October-November), dead fledglings provide a stable and consistent sample, ensuring a sufficient number of birds each year. The sampled birds belong to the same cohort and exhibit similar age, size, and health status. This uniformity minimizes bias, thereby enhancing the consistency and reliability of the data (Rodríguez et al., 2024). Dead fledglings are relatively easy and cost-effective to collect within the framework of existing rescue campaigns already implemented (~30 years) in the main archipelagos with breeding colonies.

Adult birds are excluded from the current monitoring program for several reasons. First, adult carcasses are rarely found, their availability is unpredictable, and their mortality can be caused

by a wide range of factors. Second, the timing of collection within the year significantly impacts their plastic content, as adults transfer recently ingested plastics to their offspring during feeding, clearing out >80% of their plastics at the end of breeding (Rodríguez et al., 2025; Fig. 3). Thus, breeders Cory’s shearwaters during and after chick-rearing have not been recommended as indicators of plastic, as their concentrations may underestimate adult plastic ingestion and obscure temporal patterns (Rodríguez et al., 2025).

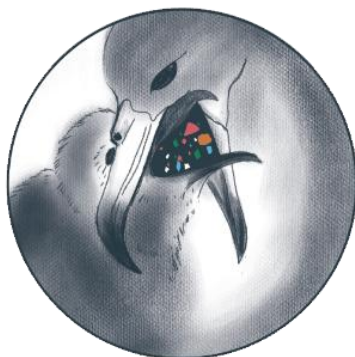


Figure 3. Representation of intergenerational transfer of plastics from adult breeders to their offspring during the chick-rearing period. The plastics transferred are the most recent ones regurgitated from the first section of the stomach, the proventriculus. (Design by @LapaBrava)

Overall, birds collected from different periods of the annual cycle (wintering grounds, migration, pre-laying incubation, and chick rearing), different age groups (offspring, fledglings, juveniles, immatures, adult or breeders), and various collection sources (beached, bycatch, victims of light pollution, predation, etc.) should not be combined to address temporal trends of plastic contamination. Yet, if comparisons are made without accounting for sample type, the conclusions drawn from the statistical analyses should be interpreted with caution.

2.3.2. Survey sites

The monitoring program can be implemented in areas where a significant number of dead fledglings are found each fledging season. While being mindful of available human resources and storage capacity, it is not necessary to collect birds from every island within archipelagos that contain multiple islands. Especially since no significant differences in plastic ingestion have been observed in different breeding populations (Rodríguez et al., 2024; Sobrino Monteliu et al., 2025). Overall, the focus should be on collecting a representative sample by prioritizing practical considerations for effectively and economically gathering and preserving the target number of deceased fledglings.

2.3.3. Survey frequency and number of samples

Dead fledglings of Cory’s shearwaters would be collected annually during the take-off period (October to November), a phase when they are exposed to artificial light pollution from urban environments upon departing from their burrow nests in the various archipelagos of Macaronesia (Atchoi et al., 2021). Ongoing rescue campaigns aimed at rescuing disoriented birds in areas impacted by light pollution can support the additional collection of corpses. The deceased birds will then be frozen (at -20°C) until a trained team can carry out the necropsies.

A **minimum sample of 40 fledglings per year** would be required for each assessment area/subregion. Yet, it is not necessary to sample an equal number of fledglings from each sex, as analyses of over a thousand birds demonstrated no disparities in plastic ingestion between males and female fledglings (Rodríguez et al., 2024).

2.4. Sampling Strategy

2.4.1. Field sampling

In the Azores, a rescue campaign called ‘SOS Cagarro’ was launched in 1995 and is coordinated by the Regional Government of the Azores. Due to its long existence and great efforts, the local population is well aware of the campaign and actively participates in the rescue of grounded birds. On each island, the work involved in the campaign is coordinated by the RGA, which guarantees the collection of live and injured shearwaters (which are sent to recovery centers or contracted clinics), as well as the vast majority of dead ones. This campaign has led to a high percentage of successful rescues (~80,000 since 1995, ranging between 3,000 to 10,000 fledglings rescued per year) which are released into the ocean after being tagged (Fig. 4). Yet, despite significant efforts, some mortality still occurs, with rates of approximately 6% in the Azores (Fontaine et al., 2011).

Under the current structure of the monitoring program in the Azores, the carcasses of Cory’s shearwaters are stored at the Environmental and Climate Action Services of each island or at the Wild Birds Recovery Centers. These services/centers are equipped with freezers (-20°C) to store as many samples as possible. Furthermore, during the collection of deceased birds, they are placed in clearly labeled bags to ensure accurate sorting during sample processing. At the end of each campaign, all collected carcasses are centralized on the island of Faial, where the RGA service, responsible for processing samples and data analysis, is based. As a result, it is always necessary to ensure their shipment from the other islands, either by air or sea. In the Azores, maritime transport of samples is only carried out within the central group of islands in the archipelago. It is guaranteed through a protocol between the RGA and the local maritime passenger traffic company, thus incurring no associated costs.

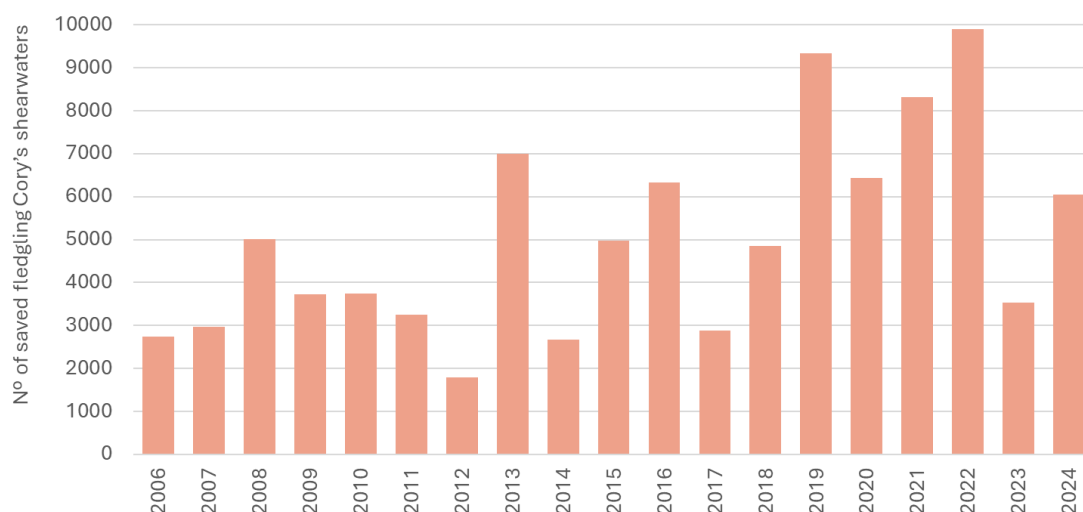


Figure 4. Graph showing the quantity of fledgling Cory’s shearwaters that were saved and released between 2006 and 2024 in the Azores archipelago through the “SOS Cagarro” rescue campaign. Fledglings become attracted to sources of artificial light at night leading them away from their natural habitats. Governmental institutions, conservation organizations, and researchers have been working in many affected areas to raise awareness about these issues. (Source: Regional Directorate for Maritime Policies, Regional Government of the Azores)

2.4.2. Authorizations for animal handling

The local bird collection work should take place during rescue campaigns, either by certified governmental staff (e.g., Nature Wardens, assigned to the Environment and Climate Action Services of the RGA) or, when necessary, with the participation of volunteers, NGOs, or research groups. All legal requirements of the local collection area must be met, namely obtaining the necessary licenses for animal handling (e.g., license assigned for the handling of wild animals under the scope of Regional Legislative Decree nº15/2012/A, April 2nd), adhering to guidelines from regional/local veterinary officers and securing licenses for accessing Protected Areas. Moreover, anyone holding a license to manage wild birds must also comply with the guidelines set by the regional veterinary office. The measures imposed by the Avian Influenza Surveillance Plan must be complied with, too. It is also recommended that the local authorities promote regular training on seabird rescue, recovery, and treatment to equip the technical teams, veterinarians, and other stakeholders with the necessary skills.

2.4.3. Dissection method and stomach analyses at the laboratory

Prior to the necropsy, the total weight of the birds needs to be recorded. The necropsy of Cory’s shearwaters should follow the well-established and standardized protocol outlined by van Franeker (2004), as detailed in the ‘CEMP Guidelines for Monitoring and Assessment of plastic particles in stomachs of fulmars in the North Sea Area’. During the necropsy of Cory’s shearwaters, both stomach compartments (proventriculus and gizzard) should be opened and examined for plastic ingestion. For this bioindicator, plastics will be recovered by filtering the stomach contents through a 0.9 mm mesh to guarantee that all plastics with 1 mm are also recovered. Then, plastic items can be separated from the organic matter and cleaned with water using metal tweezers. The plastics recovered will be dried at room temperature conditions before sorting.

2.4.4. Plastic litter classification

Monitoring litter ingested by fledgling Cory’s shearwaters will focus exclusively on plastics, as this is the type of debris ingested by the species in the breeding regions (Rodríguez et al., 2012; Navarro et al., 2023; Rodríguez et al., 2024; Sobrino-Monteliu et al., 2025), and non-breeding region (Colabuono et al., 2009; Petry et al., 2009; Baes et al., 2024; Gallo et al., 2024), at least so far. The plastic items will be classified based on their morphology, following the recommendations made by Provencher et al. (2017):

- **Plastic pellets**, also known as nurdles or industrial plastics
- **Hard plastic fragments** (remnants of indistinguishable larger objects)
- **Threadlike items** (remnants of ropes and nets)
- **Sheetlike items** (plastic bags, packaging)
- **Foam plastics** (Styrofoam, synthetic sponges)
- **Others** (e.g., rubber items)

2.5. Quality Assurance and Control (QA/QC)

To guarantee that the Quality Assurance procedures are achieved, data collection will be carried out by trained personnel following the standardized and well-established protocol defined in the current CEMP guidelines regarding the monitoring strategy (see section 2.3) and sampling strategy (see section 2.4). Additionally, to guarantee Quality Control of the information this bioindicator will provide, data submission to the responsible Cory’s shearwater leader from the OSPAR expert group should follow the information provided in Annex A (see section 3.1). Then, before carrying out assessments and reporting to OSPAR ODIMS, the responsible Cory’s shearwater leader will review the submitted data to check for errors and validate if the information submitted is the one required. Assessments for each subregion must be based solely

on data from fledglings that have died after leaving the nest. Thus, this bioindicator analysis will exclude data from other age groups (adults, immatures, or chicks). Moreover, a minimum sample size of 40 individuals per year and subregion is recommended, with data collected from both stomach sections (proventriculus and gizzard). Smaller sample sizes per year/subregion may be accepted if they meet minimum quality standards, ensuring statistical robustness for reliable assessments. Periodic expert group meetings will flag potential data issues and identify more stringent quality control protocols if necessary.

2.6. Data reporting, handling and management

The dataset generated from Cory’s shearwater monitoring will be managed by Portugal (Azores subregion). This dataset should be submitted by Contracting Parties on a biennial basis, meaning two years after the collection of samples. The format in which the report will be made is yet to be determined in consultation with the OSPAR Secretariat, but it may follow the format outlined in Annex A. The metadata will be made available in the ODIMS in compliance with INSPIRE standards.

3. Assessment

3.1. Data acquisition

The level of detail that the monitoring program can assume in each region will be defined according to the logistical constraints, both in terms of economic and human resources. Being aware of this, two levels of data collection are proposed: the first of which is mandatory, and the second is strongly recommended for long-term assessments (Fig. 5; Annex A).

3.1.1. First level:

1. **Frequency of animals having plastic in their stomachs.** The percentage frequency of occurrence (hereafter %FO) of plastic ingestion at the population level for Cory’s shearwaters is determined by evaluating the presence or absence of ingested plastics in each bird. The %FO is calculated by dividing the total number of fledglings found with plastics in the stomachs (both the proventriculus and gizzard) by the total number of birds sampled per year (including those in which no plastic was found in the stomachs). This can be evaluated for each subregion and then averaged across the entire assessment area.
2. **The number of plastic items being ingested per bird.**
3. **Total mass of all plastics ingested per bird.** This metric should be measured using a laboratory analytical balance with an accuracy of ± 0.0001 g.

Note: The number and mass of plastics can be reported separately for each stomach section (the proventriculus and gizzard), and then by providing a total value per bird for both metrics. Recording values by stomach section allows for more effective comparisons with other monitoring programs targeting other seabird species implemented as bioindicators, particularly if those other programs only include information from one of the stomach sections.

3.1.2. Second level (recommended):

4. **The number of different morphologies being ingested and their sizes.** Whenever possible, it would be valuable to report a second level of information that includes the sizes and morphologies of the different plastics. For both metrics, it is highly

recommended to report the distribution of plastic morphologies across the five general categories mentioned in section 2.4.4 (hard plastic fragments, threadlike items, sheetlike items, plastic pellets, and other plastic debris). The proportion of plastic morphologies will support the identification of specific contamination sources (Rodríguez et al., 2024). In case there is insufficient time or human resources available to complete this detailed assessment immediately, properly storing the stomach contents will allow for this sorting to be performed at a later stage.

3.1.3. Third level (optional):

5. **The size of each plastic item.** Additionally, whenever possible, each item will be measured by its longest dimension and classified into the following size categories: large microplastics (1–5 mm), mesoplastics (>5–25 mm), and macroplastics (>25 mm) (Galgani et al., 2023).

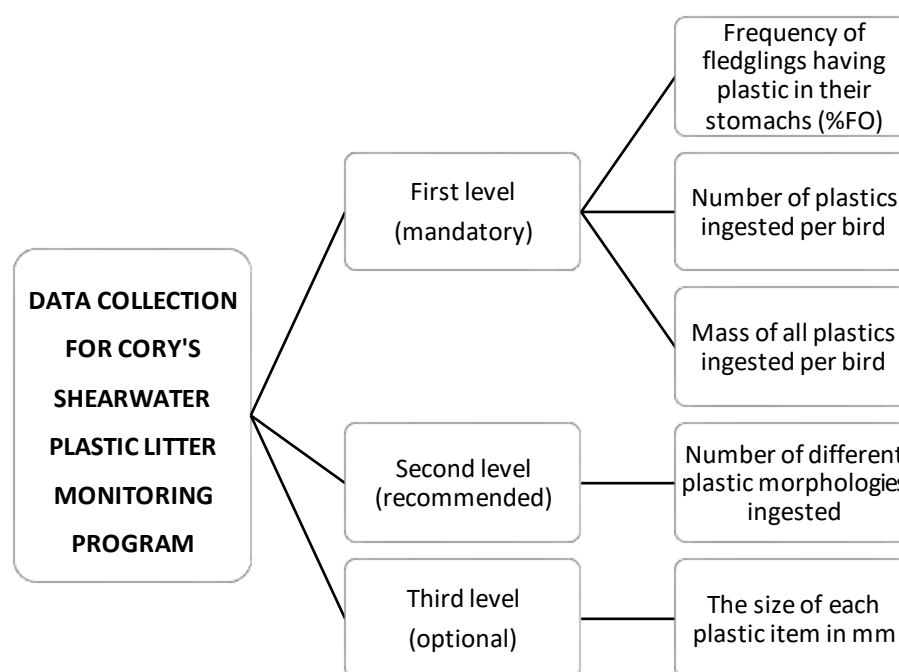


Figure 5. Levels of data collection proposed for the Cory’s shearwater plastic litter monitoring program.

3.2. Preparation of data

The data will be obtained using unique monitoring methods tailored specifically to the context of this monitoring. Consequently, there is no requirement for additional data standardization or normalization, as the methodologies themselves will inherently ensure consistency and relevance within their intended framework.

3.3. Assessment criteria – fledglings Cory’s-TV

The Cory’s shearwater threshold value, hereafter Cory’s-TV, is defined as follows (Rodríguez et al., 2024); “No more than 20% of Cory’s shearwater fledglings have > 4 plastic particles in their proventriculus and gizzards in a sample size of 200 birds or more, within a period of at least five consecutive years (on average 40 birds per year), collected dead per assessment area/subregion during the fledging season”.

Note that the cut-off option was selected as the most appropriate method for defining the threshold value (TV) determined using percentile values from the chosen baseline dataset

(Werner et al., 2020). The calculations involved selecting a low percentile from a large baseline dataset by following a similar approach used in the beach litter compartment (van Loon et al., 2020). This was due to the lack of a near-pristine foraging area for Cory’s shearwaters from which it would have been able to select a high percentile as a limit value.

The limit value for the Cory’s-TV (4 plastics/birds) was set to the 25th percentile since the other percentiles tested (10th, 15th, and 20th) were too close to a zero-litter option, making such a target unrealistic (Rodríguez et al., 2024). The variable ‘number’ was selected for this threshold since the result of the mass trend model (using eight years of data and >1,000 fledglings) showed that the fluctuations in the ‘mass’ data cannot be explained by the temporal variable ‘year’. The 20% exceedance limit proposed corresponds to an 80% confidence that the proposed threshold is reached (see Supplementary Material in Rodríguez et al., 2024). Finally, the minimum sample of 40 fledglings collected dead per year during the fledging season was established following current recognized guidelines and recommendations for monitoring plastic ingestion in seabirds in a standardized way (van Franeker and Meijboom, 2002; Provencher et al., 2019; Savoca et al., 2022; Galgani et al., 2023). Finally, the selected minimum data aggregation period aligns with the reporting requirements for OSPAR and the EU Technical Group on Marine Litter. However, this period may be extended to six years to match the timeframe used for MSFD reporting. The analysis of the current dataset shows that in all recent five-year periods, more than 20% of fledglings consistently exceed the established limit of 4 plastic items (Fig. 6). While the TV was established based on the quantified number of plastics, it is crucial to underscore the necessity of also documenting the mass of plastics, as outlined by various policies (e.g., EC, 2017; OSPAR, 2009).

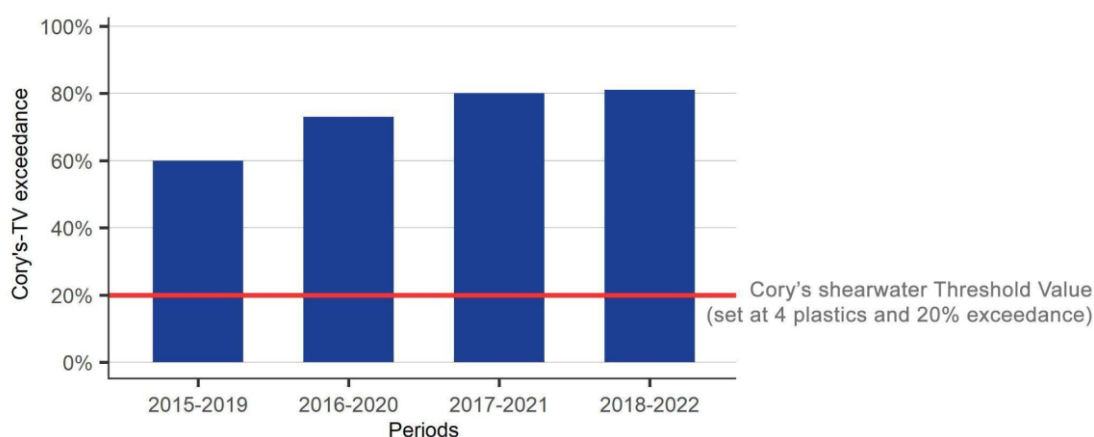


Figure 6. Cory’s-TV assessment of fledglings collected in the Azores over eight years. Data is aggregated and visualized in five-year intervals moving one year at a time. The horizontal line indicates Cory’s threshold value (Cory’s-TV), which is set to 4 plastic items and a maximum exceedance of 20%. This threshold corresponds to an 80% confidence level that Cory’s-TV has been reached.

3.4. Trend analysis

Generalized Additive Mixed-effect Models (GAMMs) were used to investigate temporal trends of plastics ingested by Cory’s shearwaters between 2015 and 2022 for two response variables: the ‘number’ and ‘mass’ of plastics. The analyses were performed in R (R Core Team, 2021). A Negative Binomial (NB) distribution with a log link function was selected for the GAMM applied

to investigate patterns in the ‘number’ of plastics, while a Tweedie distribution was applied to analyze the variable ‘mass’ of plastic (Rodríguez et al., 2024). Year was treated as a factor in both models, while the variable ‘sex’ was included in the fixed part of the models. The variable ‘island’ was incorporated as a random effect to avoid pseudoreplication.

The results of the trend models indicate an increase in the number of plastics found in Cory’s shearwater stomachs between 2015 and 2022, with the model explaining 27.2% of the variation in the ingested items over time (Fig. 7). On the contrary, the plastic mass was not a suitable metric for determining plastic temporal patterns by using Cory’s shearwaters. The plastic mass model explained only 3.52% of the variation in the ingestion of plastics over the years, did not show any temporal trend, and the 95% confidence intervals had greater uncertainty in the estimates provided compared to the model applied to the number of plastics (see Rodríguez et al., 2024 for more details).

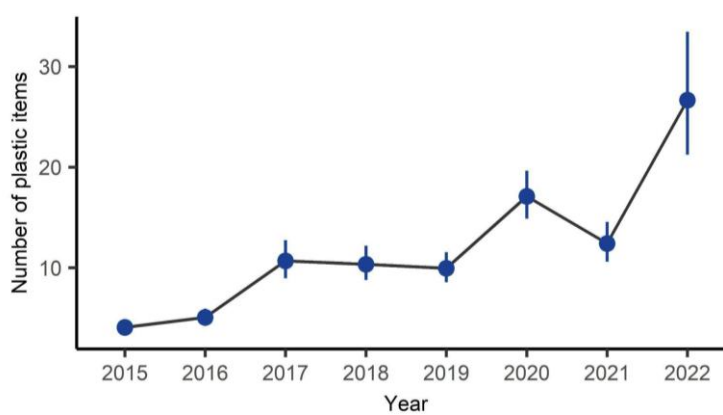


Figure 7. Temporal trend obtained for the number of plastics ingested by Cory’s shearwaters fledglings between 2015 and 2022.

Overall, to analyze plastic temporal trends using Cory’s shearwater fledglings as a bioindicator, the following general equation should be applied: $\text{NumberPlastic}_{ij} = \text{Intercept} + f\text{Year}_{ij} + a_i + \epsilon_{ij}$. In this equation, the response variable represents the ‘number’ of plastic per bird, including both stomach sections on fledgling i at the island j . The term a_i is the random intercept for i (where f denotes a factor variable), which is assumed to follow $\sim N(0, \sigma_{\text{island}}^2)$ and the ϵ_{ij} are the residuals, which are assumed to follow $\sim N(0, \sigma^2)$. The R package that should be used to run this analysis is the `mgcv` with its function `gam` (Wood, 2017).

The data collected in the Azores between 2015 and 2022 involved a great deal of effort from the local authorities and the scientific team, enabling the analyses of a sample of 1,030 fledglings from multiple islands. The mixed models were used to prevent a potential pseudoreplication in this dataset (meaning that birds from the same island may be correlated, leading to biased results). However, for future analyses, if the data is not collected from birds from several islands of the same archipelago, the statistical models applied to assess temporal trends should be adjusted to simpler Generalized Additive Models (GAMs).

3.5. Presentation of assessment results

The target audience of Cory’s shearwater assessment results includes administrations responsible for reporting on the Marine Strategy Framework Directive, as well as the scientific community specialising in marine litter, seabirds, and ecological damage. The communication of findings should prioritise peer-reviewed scientific papers and periodic assessments to ensure rigor and relevance to these stakeholders.

4. Change Management

The ingestion of litter and microlitter by Cory’s shearwaters in the Azores is used in the reporting of criterion D10C3 (MSFD) and integrated into the MSFD PT-MO-D10-B monitoring program. The PT-MO-D10-B was established in 2021 and is coordinated by the Azores Regional Government. Considering the species also breed in other regions, engaging stakeholders from those is recommended to improve the enlarged use of Cory’s shearwaters as an indicator of floating plastics in a wider area of the NE Atlantic. This is particularly relevant because the birds from different archipelagos use different foraging areas during the breeding season and thus represent different levels and sources of plastic contamination (Rodríguez et al., 2024). Additionally, if sufficient carcasses are available, the current monitoring program could be extended to encompass other shearwater species breeding in European countries. Integrating long-term bioindicators is crucial for a global plastic pollution observing system, ensuring their effectiveness and inclusivity through harmonized and standardized methods supported by regional collaboration, data sharing, and open science practices (Savoca, et al., 2025), as proposed in the current CEMP guidelines. The follow-up on the common indicator assessment should be agreed upon within the ICG-ML and the Fulmar EG. Nevertheless, it is proposed that this assessment should be carried out on the ‘Marine Litter ingested by Seabirds’ EG (taking example the EG for marine litter ingested by sea turtles), aggregating both fulmar and Cory’s shearwater experts. Yet, Cory’s shearwater assessment report will be presented by contracted parties in OSPAR region V.

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ANNEX A - Description of Cory’s Shearwaters data submission

To standardize data submission to OSPAR for international assessments, the specific variables required must be submitted in a strictly standardized format to ensure consistency and uniformity across all contributions.

A. General variables to record for level 1 (mandatory, see CEMP section 3.1.1):

1. ID code made with Year + subregion + sequential number (i.e., 2024_AZO_01)
2. Country (e.g., Portugal)
3. Assessment area or subregion (e.g., Azores, Madeira)
4. Island (mandatory for data collected in archipelagos)
5. Species_ScientificName (e.g., Calonectris borealis)
6. Species_CommonName (e.g., Cory’s shearwater)
7. Year of collection
8. SampleDate in the following format YYYYMMDD
9. Type of sample (light-pollution casualty, beached bird, bycatch, other (specify) unknown)
10. Potential cause of death (collision, road-killed, extreme weather, domestic predator, oil pollution, disease, other (specify), unknown...)
11. Age (FLEDG = fledglings, AD = adult breeder, CK = chick, OTH = others such as immatures or juveniles, UNK = unknown) Note: this bioindicator only uses fledglings data, so please, do not report data from other ages
12. Total bird weight in grams

Information to obtain through a necropsy:

13. Sex (M = male, F = female, unk = unknown)
14. Total number of plastics per bird (combining information from the proventriculus and the gizzard) – TOTAL n_plastics
15. Total mass of plastics per bird (in grams to 4th decimal, combining information from the proventriculus and the gizzard) – TOTAL g_plastic

B. General variables to record for level 2 (recommended, see CEMP section 3.1.2):

16. Number of industrial plastic pellets per bird - n_IND
17. Number of hard plastic fragments per bird - n_frag
18. Number of threadlike items per bird - n_thr
19. Number of sheetlike items per bird - n_she
20. Number of foam plastics per bird - n_foa
21. Number of other plastics per bird - n_other

C. General variables to record for level 3 (optional, in a different Excel sheet, see CEMP section 3.1.3):

22. Maximum length size (mm) of each plastic item per ID

Annex II – Student contribution to the thesis and research dissemination

II.1. Personal contribution to each of the scientific chapters

CHAPTER II contribution: YR personally conducted the majority of the necropsies (> 1,000 birds) and assisted other collaborators who sporadically helped during the laboratory work. YR also supported the DRPM by providing materials, protocols, and labels necessary for the collection of fledglings during the take-off season campaign. YR sorted all the plastics included in this study and entered the data into a database. YR conducted the statistical analysis and drafted the original manuscript, thoughtfully incorporating suggestions from co-authors.

CHAPTER III contribution: YR conceived the idea for this work. YR participated in the collection of some of the adult's corpses for which no specific collection campaign exists. She conducted the laboratory work necessary for these analyses. YR introduced the data and conducted the statistical analysis and drafted the original manuscript, thoughtfully incorporating suggestions from co-authors.

CHAPTER IV contribution: YR conceived the idea for this work, and gathered the information into a comprehensive database. YR conducted the statistical analysis and drafted the original manuscript, thoughtfully incorporating suggestions from co-authors.

CHAPTER V contribution: YR conceived the idea for this work with the supervisor. She defined the criteria for the index developed, which were then discussed and improved with the co-authors. YR conducted the statistical analysis and drafted the original manuscript, thoughtfully incorporating suggestions from co-authors.

CHAPTER VI contribution: YR conceived the idea for this work with the supervisor, engaged with all relevant stakeholders, and diligently compiled the information into a comprehensive database. YR conducted the statistical analysis and drafted the original manuscript, thoughtfully incorporating suggestions from co-authors.

II.2. Publications included in the thesis

II.2.1. Peer-reviewed by date of publication

Rodríguez, Y., Silva, M.A., Pham, C.K. and Duncan, E.M., 2023. Cetaceans playing with single-use plastics (SUPs): A widespread interaction with likely severe impacts. *Marine Pollution Bulletin*, 194, p.115428. <https://doi.org/10.1016/j.marpolbul.2023.115428>

Rodríguez, Y., Rodríguez, A., van Loon, W.M., Pereira, J.M., Frias, J., Duncan, E.M., Garcia, S., Herrera, L., Marqués, C., Neves, V. and Domínguez-Hernández, C., 2024. Cory's shearwater as a key bioindicator for monitoring floating plastics. *Environment International*, 186, p.108595. <https://doi.org/10.1016/j.envint.2024.108595>

Rodríguez, Y., Rodríguez, A., Pereira, J.M. and Pham, C.K., 2025. Plastics reset in an adult Procellariiform seabird species during the breeding season. *Marine Environmental Research*, p.106939. <https://doi.org/10.1016/j.marenvres.2024.106939>

Rodríguez, Y., Vandeperre, F., Duncan, E.M., Machete, M. and Pham, C.K., 2025. An index to differentiate megafauna entangled in operational fishing gears from abandoned, lost, or otherwise discarded fishing gears. *Science of The Total Environment*, 971, p.178950. <https://doi.org/10.1016/j.scitotenv.2025.178950>

Rodríguez, Y., Vandeperre, F., Parra, H., Machete, M., Fontes, J., Silva, M., & Pham, C. K. (accepted). 2026. Marine debris entanglements and associated impacts in megafauna across the open NE Atlantic Ocean. *Endanger. Species Res.* <https://doi.org/10.3354/esr01485>

II.2.2. Policy contribution

Rodríguez, Y., Garcia, S., & Pham, C. K. 2025. CEMP Guidelines for monitoring and assessment of plastic litter ingested by fledglings of Cory's shearwaters (*Calonectris borealis*) in OSPAR Region V. OSPAR Agreement 2025–16. <https://www.ospar.org/documents?v=63717>

II.3. Conferences, meetings and other communications as first author

II.3.1. Oral presentations in conferences or symposiums

6th–8th July 2022: Entwined in Plastic: Investigating Entanglements in Marine Litter of Megafauna in the NE Atlantic

Presented at the VIII International Symposium on Marine Science (ISMS 2022) during the BAMAR session in Gran Canaria, Spain. <https://isms-canarias.com/en-isms>

3rd–4th November 2022: Impactos do Plástico no Mar dos Açores

Presented at Bluefest: Ciência e Educação para a Economia Azul, Azores, Portugal. <https://bluefest.pt/>

7th-8th March 2023: Cory's Shearwaters as a Key Species to Monitor Oceanic Plastics in Hotspots of Marine Litter in the NE Atlantic

Presented at Marlice Islands 2023: International Forum on Marine Litter & Circular Economy in Island Environments, during the session dedicated to hotspots and ecosystem impacts, Tenerife, Spain.

10th-12th July 2024: Investigating Persistent Organic Pollutants in Cory's Shearwater: A Key **Rodríguez, Y., Hernández-Borges, J., Martins, I., & Pham, C. K.**

Presented at the IX International Symposium on Marine Science (ISMS 2024) during the BAMAR session in Valencia, Spain. <https://ismsvalencia.es/>

2nd–6th September 2024: Cory's Shearwater as a Key Bioindicator of Plastic Contamination in NE Atlantic Hotspots of Marine Litter

Presented at the XVI International Seabird Group Conference, organized by the research group ECOTOP (Ecology and Conservation of Top Predators) in Coimbra, Portugal. <https://www.uc.pt/events/coimbra-seabird-conference/>

II.3.2. Oral presentations in projects

16th April 2024: Cory's shearwater as bioindicator for D10C3 and research in the Azores to support D10C4.

Presented at Projecto LIFE SeaBil – Monitorização do impacto do lixo marinho invited by SPEA (<https://spea.pt/>) to present her research work at the 2nd transnational workshop of the LIFE SeaBil project (<https://pt.lifeseabil.eu/>), which took place in Peniche, at the CETEMARES building of the Polytechnic Institute of Leiria, and was attended by SeaBil project partners from Spain and France, as well as other guests from various universities and other organisations.

II.3.3. Oral presentations in policy events

18th to 21th June 2024: Cory's shearwater – marine litter bioindicator for region V.

Presentation at first full meeting of the Intersessional Correspondence Group on Marine Litter in 2024 (ICG-ML(1) 2024).

14th Outubro 2024: Cagarros - bioindicador de microplásticos nos açores.

Opening session of the 30th edition of the SOS Cagarro Campaign at the Center for Contemporary Arts, an initiative promoted by the Regional Secretariat for the Sea and Fisheries, through the Regional Directorate for Maritime Policies. Presided over by Her Excellency the Minister of Environment and Energy, Maria da Graça Carvalho, the event was attended by the Regional Secretary for Tourism, Mobility, and Infrastructure, Berta Cabral, with contributions from the Regional Secretary for Environment and Climate Action, Alonso Miguel, the Regional Director for Maritime Policies, Rui Martins.

II.3.4. Conference posters

18th to 23th September 2022: Entwined in plastic: Investigating entanglements in marine litter of megafauna in the NE Atlantic.

7th International Marine Debris Conference (<https://7imdc.org/main>) in Busan, Republic of Korea.

II.3.5. Workshops

18th-20th November 2022: Facilitating a workshop on necropsies of marine turtles and seabirds in collaboration with the Regional Directorate for Maritime Policies and researchers from Okeanos.

17th February 2025: Facilitating a workshop on necropsies of seabirds for a group of veterinarian students for the Institute of Biomedical Sciences Abel Salazar (ICBAS) of the University of Porto.

II.4. Other peer-reviewed papers published during the thesis

II.4.1. Peer-reviewed papers in the scope of the thesis

Rodríguez, Y., Vandeperre, F., Santos, M.R., Herrera, L., Parra, H., Deshpande, A., Bjorndal, K.A. and Pham, C.K., 2022. Litter ingestion and entanglement in green turtles: an analysis of two decades of stranding events in the NE Atlantic. *Environmental Pollution*, 298, p.118796. <https://doi.org/10.1016/j.envpol.2022.118796>

Fackelmann, G., Pham, C.K., **Rodríguez, Y.**, Mallory, M.L., Provencher, J.F., Baak, J.E. and Sommer, S., 2023. Current levels of microplastic pollution impact wild seabird gut microbiomes. *Nature ecology & evolution*, 7(5), pp.698-706. <https://doi.org/10.1038/s41559-023-02013-z>

Savoca, M. S., Abreo, N. A., Arias, A. H., Baes, L., Bains, M., Bergami, E., Brander, S., Canals, M., Choy, C. A., Corsi, I., De Witte, B., Domit, C., Dudas, S., Duncan, E. M., Fernández, C. E., Fossi, M. C., Garcés-Ordóñez, O., Godley, B. J., González-Paredes, D., González Carman, V., Hamilton, B. M., Hardesty, B. D., Hong, S. H., Kahane-Rapport, S., Kashiwabara, L. M., Lacerda, M. B., Luna-Jorquera, G., Manno, C., Nelms, S. E., Panti, C., Pérez-Venegas, D. J., Pham, C. K., Provencher, J. F., Purca, S., Rashid, H., **Rodríguez, Y.**, Sparks, C., Sun, C. J., Thiel, M., Tsangaris, C., & Santos, R. G. 2024. Monitoring plastic pollution using bioindicators: a global review and recommendations for marine environments. *Environmental Science: Advances*. <https://doi.org/10.1039/D4VA00174E>

II.4.2. Book chapters in the scope of the thesis

Nelms, S.E., Clark, B.L., Duncan, E.M., Germanov, E., Godley, B.J., Parton, K.J., Pham, C.K. and **Rodríguez, Y.**, 2023. Plastic pollution and marine megafauna: Recent advances and future directions. *Plastic Pollution in the Global Ocean*, pp.97-138.

II.4.3. Other peer-reviewed papers published during the thesis

Peres dos Santos, R., **Rodríguez, Y.**, Sears, R., Magno, R. and Castilho, R., 2025. Tour Operators as a Tool to Improve Information on Data-Deficient Cetacean Species. *Aquatic Conservation: Marine and Freshwater Ecosystems*, 35(2), p.e70067. <https://doi.org/10.1002/aqc.70067>

Rodrigues, C., **Rodríguez, Y.**, Frias, J., Carriço, R., Sobral, P., Antunes, J., Duncan, E.M. and Pham, C.K., 2024. Microplastics in beach sediments of the Azores archipelago, NE Atlantic. *Marine Pollution Bulletin*, 201, p.116243. <https://doi.org/10.1016/j.marpolbul.2024.116243>

Duncan, E. M., Vieira, N., González-Irusta, J. M., Dominguez-Carrió, C., Morato, T., Carreiro-Silva, M., Jakobsen, J., Jakobsen, K., Porteiro, F., Schläpfer, N., Herrera, L., Ramos, M., **Rodríguez, Y.**, Pereira, J. M., Fauconnet, L., Rodrigues, L., Parra, H., & Pham, C. K. 2023. Predicting the distribution and abundance of abandoned, lost or discarded fishing gear (ALDFG) in the deep sea of the Azores (North Atlantic). *Science of the Total Environment*, 900, p.166579. <https://doi.org/10.1016/j.scitotenv.2023.166579>

Pham, C.K., Estevez, S.G., Pereira, J.M., Herrera, L., **Rodríguez, Y.**, Domínguez-Hernández, C., Villanova-Solano, C., Hernández-Sánchez, C., Díaz-Peña, F.J. and Hernández-Borges, J., 2023. Three-dimensional evaluation of beaches of oceanic islands as reservoirs of plastic particles in the open ocean. *Science of the Total Environment*, 900, p.165798. <https://doi.org/10.1016/j.scitotenv.2023.165798>

Cuesta-García, M., Rodríguez, A., Martins, A.M., Neves, V., Magalhães, M., Atchoi, E., Fraga, H., Medeiros, V., Laranjo, M., **Rodríguez, Y.**, Jones, K., Bried, J. 2022. Targeting efforts in rescue programmes mitigating light-induced seabird mortality: First the fat, then the skinny. *Journal for Nature Conservation*. 65, 126080. <https://doi.org/10.1016/j.jnc.2021.126080>

II.5. Some outreach and media coverage of the thesis

II.5.1. Chapter II

National news: Cagarro reconhecido como bioindicador de poluição marinha por plásticos (Source: **Sic Notícias**)

National newspaper news: Mais de 90% das crias de cagarro têm plásticos no estômago quando saem do ninho (Source: **Público**)

National newspaper news: Na altura em que abandonam o ninho, 90% dos cagarros já têm o estômago cheio de plástico, mostra estudo do Instituto Okeanos (Source: **Visão**)

National blog: Mais de 90% das crias desta ave têm plásticos no estômago mesmo antes de saírem dos ninhos (Source: **Wilder**)

Regional newspaper news: Portugal e Espanha vão poder contar com os cagarros para servir de bioindicador do lixo marinho no Atlântico Nordeste (Source: **Correio dos Açores**)

Regional television news: Portugal e Espanha vão contar com cagarros como bioindicador de lixo marinho (Source: **RTP Açores**)

Regional newspaper news: Cagarro seleccionado bioindicador do lixo marinho no Atlântico Norte (Source: **Diário dos Açores**)

International blog: Más del 90 % las crías de pardela de Canarias y Azores tiene plástico en el estómago (Source: **Efe Verde**)

International newspaper news: Más del 90% de las crías de pardela de Canarias tienen plástico en el estómago (Source: **El Diario**)

International news: Seleccionan a la pardela cenicienta como bioindicador para vigilar la acumulación de plásticos en el Atlántico norte (Source: **CSIC**)

International news: La pardela cenicienta, seleccionada como bioindicador para vigilar los plásticos en el Atlántico norte (Source: **Europa Press**)

International television news: Más del 90 % de las crías de pardela de Canarias tiene plástico en el estómago (Source: **Radio Television Canaria – RTVC**)

International news: Pardela cenicienta: el ave que sirve como bioindicador para monitorizar la acumulación de plásticos en el Atlántico Norte (Source: **Nova Ciencia**)

International television news: Más del 90% de los juveniles de pardela cenicienta en Canarias tienen plástico en sus estómagos (Source: **La Sexta Televisión**)

International newspaper: Más del 90 % de las crías de pardela de Canarias y Azores tiene plástico en el estómago (Source: **La Provincia**)

International news: Más do 90% das crías de pardela cincenta teñen plásticos no estómago (Source: **gCiencia**)

International news: Más del 90 % las crías de pardela de Canarias y Azores tiene plástico en el estómago (Source: **InfoBae**)

International news: Dato alarmante: más del 90% de las crías de pardelas de Canarias y Azores tienen plástico en el estómago (Source: **Canal26**)

International news: La pardela cenicienta, un bioindicador de plásticos marinos en el Atlántico (Source: **Cinco Noticias**)

International news: Más del 90% de las crías de pardela cenicienta en Canarias tienen plástico en el estómago (Source: **La Vanguardia**)

International news: Seleccionan a la pardela cenicienta como bioindicador para vigilar la acumulación de plásticos en el Atlántico norte (Source: **Siete días Marbella**)

International news: Más del 90 % de los juveniles de pardela cenicienta en Canarias tienen plástico en sus estómagos (Source: **Agencia Sinc**)

International news: Un estudio en el que participa la ULL propone a la pardela cenicienta como bioindicador de plásticos marinos en el Atlántico Norte (Source: **ULL**)

International news: Más del 90% las crías de pardela de Canarias y Azores tiene plástico en el estómago (Source: **Diario de Canarias**)

International news: Proponen a la pardela cenicienta como bioindicador de plásticos marinos (Source: **Periodismo Ull**)

International television news: Casi 13 millones de toneladas de plástico llegan a los océanos cada año (Source: **RTVE Canarias**)

International radio interview: ‘Hoy por Hoy’ program from 01:40 (Source: **Cadena SER**)

II.5.2. Chapter V

International science blog: The Plastic Playtime Tragedy: How whales and dolphins fall victim to plastic pollution (Source: **Oceanbites, Ocean Science for Everyone!**)

II.5.3. Chapter VI

Regional newspaper news: ENSA identifica origem dos emaranhamentos marinhos (Source: **Açoriano Oriental**)

II.5.4. Other products generate during the thesis that gained attention

International policy news: Green turtles in remote Azores feeding grounds are ingesting plastic, reveals new study (Source: **Directorate-General for Environment – European Commission**)

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TD

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Yasmina Rodríguez

2025