Silvia Rao

Temporal trends of plastic ingestion and fishing gear entanglement in aquatic birds from Portugal

UNIVERSIDADE DO ALGARVE

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Silvia Rao

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Mestrado em Biologia Marinha

Supervisor:
Dr Katy Nicastro
Co-supervisor
Dr Gerardo I. Zardi

UNIVERSIDADE DO ALGARVE

Faculdade de Ciências e Tecnologia

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DECLARAÇÃO DE AUTORIA DE TRABALHO

Temporal trends of plastic ingestion and fishing gear entanglement in aquatic birds from Portugal

Declaro ser a autora deste trabalho, que é original e inédito. Autores e trabalhos consultados estão devidamente citados no texto e constam da listagem de referências incluída.

(Silvia Rao) 09.10.2020

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ABSTRACT

Improperly disposed anthropogenic litter and fishing material acutely affect wildlife species in the terrestrial and aquatic ecosystems. The foraging behaviour of birds makes them suitable to monitor changes of anthropogenic litter over time in the environment. The general aim of the thesis is to quantify and qualify species-specific changes over time due to anthropogenic litter ingestion and fishing gear entanglements. In Chapter 2, the specific objective was to test for temporal changes of anthropogenic litter ingestion in aquatic and marine birds in southern Portugal. Across the seven years (2014-2020) and out of the four bird species (total sample size of 462) analysed, *Ciconia ciconia* had the highest frequency of occurrence (61.1%), followed by *Larus fuscus* (20.8%), *Morus bassanus* (20.3%) and *Larus michahellis* (13.4%). In all species, User plastics (plastic category) was the most abundant type of material ingested and white/off clear was the predominant ingested colour. In Chapter 3, the aim intent was to test for temporal trends in fishing gear entanglements in aquatic and marine bird species over a ten year period (2010 - 2019). Of the 5843 individuals processed, 256 were affected either by fishing nets, fishing lines with or without hook. *Phalacrocorax carbo* showed a significant decrease in entanglements, whereas *Morus bassanus* showed the opposite pattern. Our findings provided an overview on temporal litter ingestion and entanglements on bird species in southern Portugal.

Keywords: Anthropogenic litter, plastic ingestion, fishing gear entanglements, birds, Portugal

RESUMO

Uma grande quantidade do plástico produzido todos os dias é descartado inadequadamente na forma de lixo antropogénico e material de pesca. Este lixo afeta gravemente as espécies na vida selvagem, tanto em ecossistemas terrestres como aquáticos.

A dispersão do lixo marinho depende de uma ampla gama de aspetos ambientais e antrópicos. As atividades humanas estão diretamente ligadas ao lixo plástico. As áreas geográficas e as condições sociais devem ser tidas em consideração, uma vez que as regiões altamente industrializadas e populosas são as mais afetadas pela poluição de plástico. Desta forma, é fundamental entender a extensão desse impacto e de como ele se modifica ao longo do tempo.

Os estudos sobre o impacto do lixo em organismos aquáticos ou de zona costeira têm vindo a aumentar e, através da análise do conteúdo estomacal de animais analisados, a quantidade e características do lixo ingerido pode ser determinada e monitorizada. O amplo comportamento na busca de alimento das aves, torna-as perfeitamente adequadas para monitorizar as mudanças ambientais ao longo do tempo.

No Capítulo 2, o objetivo foi quantificar e qualificar o impacto de lixo antropogénico usando a análise multivariada de longo prazo para observar tendências específicas da espécie ao longo do tempo em termos de abundância, massa e a cor do material ingerido. De um total de 462 aves foram analisadas neste exame, 22.7% ingeriram lixo antropogénico. Todas as quatro espécies examinadas foram afetadas pela ingestão de lixo antropogénico, predominantemente plástico. A espécie *Ciconia ciconia* foi a mais afetada pela ingestão antropogénica de lixo (61.1%) e ingestão de plástico (55.6%), seguida por *Larus fuscus* (correspondentemente 21.4% e 20.8%), *Morus bassanus* (22% e 20.3%) e *Larus michahellis* (13.4% e 13.4%), durante um período de sete anos (2014 - 2020).

As espécies também foram afetadas por itens não plásticos, principalmente vidros. A espécie *M. bassanus*, além disso, ingeriu anzóis de pesca.

Já a espécie *C.ciconia*, que geralmente busca alimento em águas rasas interiores ou costeiras, apresentou Frequências de ocorrência consistentemente mais altas em comparação com as outras três espécies. De qualquer forma, *C. ciconia*, *L. fuscus e M. bassanus* tiveram um aumento exponencial na ingestão de lixo ao longo dos anos (Frequência de ocorrência), bem como um aumento no índice de Abundância. Os resultados demonstraram que a abundância e o tipo de plástico ingerido variam substancialmente entre as espécies. Os resultados dos testes de PERMANOVA mostraram

que os anos tiveram um efeito significativo na abundância e massa de lixo ingerido por *L. fuscus* (abundância, $F_{142,137}$ = 2.29, P = 0.04; massa, $F_{143,138}$ = 13.51, P = 0.001), um efeito significativo na massa de lixo ingerido por *L. michahellis* ($F_{142,137}$ = 13.79, P = 0.0001) e o lixo ingerido pela espécie *C. ciconia* mudou ao longo dos anos em termos de abundância ($F_{51,45}$ = 2.2339, P = 0.02). Os "user plastics" (categoria de plástico) prevalecem consideravelmente sobre os outros materiais, e o branco / sem cor, foi o preferido como a cor dos itens ingeridos por todas as espécies. Independentemente das tendências temporais não significativas na análise multivariada, notam-se diferenças pronunciadas entre espécies na ingestão de plástico. Métodos padronizados foram usados para facilitar futuras comparações. Esta investigação demonstra uma quantidade recorde de existência de lixo antropogénico na dieta das espécies examinadas, e que este pode ser encontrado nas áreas onde estas espécies se alimentam.

O Capítulo 3 pretende dar uma visão geral sobre de que forma as espécies de aves registadas são afetadas por emaranhados de material de pesca nas regiões sul de Portugal (Algarve e Alentejo), entre 2010 e 2019. A intenção é compreender quanto as artes de pesca perdidas acidentalmente, ou despejadas ilegalmente, afetam as espécies de aves. O emaranhamento de animais é uma das maiores ameaças devido ao lixo plástico. A falta de informação e, acima de tudo, a escassez de registos, tornam difícil uma avaliação realista das mudanças temporais e espaciais na taxa de emaranhamento. Atualmente, não existe informação publicada sobre o enredamento de aves aquáticas em Portugal. A presente tese concentrou-se no emaranhamento das artes de pesca em 16 aves aquáticas e marinhas durante um período de dez anos (2010-2019). Dos 5.843 indivíduos, 256 foram afetados por redes de pesca, linhas de pesca com anzol ingerido ou apenas linhas de pesca. Proporcionalmente, as espécies mais afetadas pelos emaranhamentos foram *Sterna caspia* (100%) e *Larus marinus* com 50%, enquanto as menos afetadas foram Larus audouinii com 2.4%, seguida por *Gallinula chloropus* (4.2%) e *Phoenicopterus ruber* (5.9%). A mortalidade por *Calidris alpina*, *Gallinula chloropus*, *Pluvialis squatarola*, *Platalea leucorodia*, *Phoenicopterus ruber* e *Sterna caspia* foi de 100%. Foi observada uma tendência ao longo do tempo em *Phalacrocorax carbo*, onde ao longo dos anos, menos indivíduos foram afetados por emaranhamentos, enquanto que *C. ciconia* (valor P: 0.05) e *M. bassanus* (valor P: 0.01) foi encontrado um aumento significativo no número de enredamentos ao longo dos anos. Curiosamente, os resultados mostram que as diferentes espécies não apresentaram padrões consistentes em termos de percentagem de indivíduos afetados pelo emaranhamento, e daqueles que sofrem consequências letais. A espécie *P.*

carbo apresentou uma diminuição ao longo do tempo na frequência de ocorrência e taxa de liberação de 58.3%, enquanto *M. bassanus* apresentou incremento na frequência de ocorrência ao longo do tempo, apresentando uma alta taxa de mortalidade (89.7%). Ambas as espécies marinhas mostraram resultados distintos, dados por diferenças comportamentais, como técnicas de caça. Esses resultados destacam a importância crucial das avaliações multiespécies.

As observações durante este estudo descrevem uma parte real dos emaranhados na região examinada. Como o emaranhamento é caracterizado por um conjunto mais estreito de itens de plástico, em comparação com a ingestão de plástico, realizam-se ações de mitigação mais focadas. À medida que a presença de artes de pesca continua a aumentar nos ambientes costeiros e aquáticos, os nossos dados irão fornecer um registo crucial de espécies emaranhadas e uma base a partir da qual se levantam tendências a longo prazo no emaranhamento de plástico, em particular para programas de monitorização portugueses, para os quais os dados são ainda escassos.

A área de estudo desta investigação é o sul de Portugal, onde a avifauna é dominante durante todo o ano, devido à posição geográfica da Península Ibérica. A maior parte das aves encontra-se nos sistemas lagunares da Ria Formosa, que lhes fornecem alimento e abrigo. O interesse por esta região foi motivado pelo facto de ainda existirem aterros a céu aberto nesta área, que é um local turístico durante todo o ano e que é uma área de pesca intensiva, pelo que lixo antropogénico é frequente e comummente encontrado. Este estudo forneceu uma visão geral temporal da ingestão de lixo e do emaranhamento de artes de pesca em espécies de aves marinhas e aquáticas no sul de Portugal.

Palavras-chave: Lixo antropogênico, ingestão de plástico, emaranhados de artes de pesca, aves, Portugal

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C. ciconia – Ciconia ciconia

L. fuscus – Larus fuscus

L. michahellis – Larus michahellis

M. bassanus – Morus bassanus

MC – Monte Carlo

MD – Mahalanobis distance

MDS – multidimensional scaling

P. carbo – Phalacrocorax carbo

CHAPTER 1: GENERAL INTRODUCTION

Anthropogenic litter pollution is a universal threat that is jeopardising the natural environment and the functioning of ecosystems worldwide. Concerns related to the toxicity and the physical damage it may cause have increased in the last decades. Anthropogenic pollution exists in all states of matters, rendering it difficult to fully grasp the magnitude and the temporal trends of its impact. Plastic comprised the most abundant form of anthropogenic litter, it forms up to 95% of the waste that accumulates on beaches, ocean surface or seafloor (Galgani et al. 2015; Araújo et al. 2018). Scientists estimated 2013 that at least 5.25 trillion plastic fragments, of all sizes, could enter the world's oceans, which amounts to 268,940 tons of plastic particles (Eriksen et al. 2014). In particular, 60 to 80% of marine litter is plastic (Derraik 2002; Barnes et al. 2009). In 2017, plastic production reached 348 Million Tonnes (excluding PET-, PA-, and polyacrylic -fibres; PlasticsEurope 2018). From 2016 to 2017 the global and the European plastic demand increased by 13 and by 4.4 Million Tonnes respectively (PlasticsEurope 2018).

Plastic litter is differentiated in micro (< 5 mm) and macroplastic (> 5 mm), depending on its size. The vast diversity in size and shape allows it to be present across different ecosystems on this planet – in the Antarctic oceans (Obbard et al. 2014), in the deep sea (Mariana Trench; Chiba et al. 2018), in lakes, sediments, throughout the trophic levels and recently also discovered in the human body (Cox et al. 2019). 2013 it was estimated that at least 5.25 trillion plastic fragments, of all sizes could be found in the world's oceans, which amounts to 268,940 tons of plastic particles (Eriksen et al. 2014). In particular, 60 to 80% of the marine litter is plastic (Derraik 2002; Barnes et al. 2009). Still plastic waste accumulation in the sea is extremely variable (Lambert and Sinclair 2014; Moore et al. 2009).

The degradation of plastic in the sea depends on the amount of abiotic factors, such as solar heat radiation, wind, waves, water pressure and in deeper areas also on microbial communities which promote the degradation of the different polymers (Zettler et al. 2013). On the other hand, the dispersion of such litter relies on an extensive range of environmental and anthropogenic aspects. These involve physical forces such as winds, currents and coastline profiles (Law et al. 2010) and human activities such as the proximity to the cities, industrialized localities and shipping routes (Barnes et al. 2009). Highly industrialized and populated regions are the most affected by plastic pollution (Derraik 2002). Land-based plastic pollution is a considerable component (80%) of the waste entering the marine ecosystems (Gregory and Ryan

1997) while the rest (20%) are discarded fishing gears from fish vessels and cargo ships (Good et al. 2010). This lost or discarded fishing equipment, also known as “ghost fishing”, directly harm marine ecosystems by impacting fish stocks and benthic environments.

Plastic is a long-lasting material, and thus has, considerable valuable for the manufacture of supply. In the last decades, plastic consumption grew exponentially, reaching 359 million tons in 2018 worldwide (Plastics Europe 2019). The amount of produced plastic exceeds the capacity of waste management systems to dispose it appropriately or to recycle it (Jambeck et al. 2015). Even though the recycling of plastic waste in Europe has doubled since 2006, 25% of “plastic post-consumer waste” is still transported to landfills (Plastics Europe 2019). In 1999 the Council Directive 1999/31/EC declared the future closure of landfills, however, open landfills are still operational and even if well managed they lead to problems such as greenhouse gases, leachates and they affect the foraging behaviour of birds (Matejczyk et al. 2011, Council Directive 1999). Countries that comply with the requirements of the landfill restrictions (Directive 1999/31/EC) have on average a higher recycling rate. The southern, as well as the western European countries, have deposited more than 20% of their waste in landfills (Plastics Europe 2019).

Marine mammals, fishes, marine and aquatic birds ingest macro and microplastic. Different species, with different migratory and foraging behaviour, show distinct responses and impacts on plastic pollution (Tortosa et al. 2002). Ingestion can occur unintentionally, while foraging on a prey, or intentionally when material features natural food (Wilcox et al. 2015; Cadée 2002). Seabirds, shorebirds, and coastal terrestrial birds are particularly affected by anthropogenic litter (e.g. Laist 1997). Plastics float and accumulate at the surface, therefore surface-feeding seabirds tend to ingest more plastic than pursuit-diving birds (O’Hanlon et al. 2017; Poon et al. 2017a). The number of species affected is indeed rising and it is predicted that by 2050, 99% of all seabirds will experience plastic ingestion (Wilcox et al. 2015). Ingestion of material can lead to injuries in the form of internal wounds and ulcers and, gastrointestinal obstruction, which can be lethal (Puskic et al. 2020). Furthermore, birds carrying plastics in their stomach are exposed to potential toxicological effects arising from leachates, contaminants that are added during plastic manufacturing (Guo et al. 2020; Roman et al. 2019).

Plastic litter ingested by birds does not necessarily reflect the abundance of litter in the environment; nevertheless, the plastic waste is an accurate intermediary for spatial and temporal trends in the abundance and typology of plastic litter (e.g. Van Franeker et al. 2011a; Van Franeker and Law 2015). As a result, monitoring stomach contents across aquatic and marine bird species is becoming a relevant way to monitor changes in plastic pollution (Trevail et al.

2015). Depending on species and plastic typology, plastic ingestion temporal trends can differ greatly (Van Franeker et al. 2011b). Aquatic and marine bird species also incorporate plastic litter, mostly coming from fisheries, into their nest, which can lead to ingestion of pieces by their juveniles or entanglements of their limbs.

Fishing gear entanglements mostly cause the death of the animal because fishing gear predominantly gets coiled around the limbs which leads to the impossibility of movement and therefore to starvation. Occasionally the affected limb is amputated due to the necrosis of the tissue and the individual survives in case of good body condition. Fishing gear such as fishing lines and ropes are made of durable and very resistant polymers, which can last for decades in the water and harm pelagic as well as benthic organism (Laist and Wray 1995). This equipment reaches the oceans accidentally by getting lost overboard or illegally by being dumped into the water, therefore it is not only found adjacent to the coasts but also as ghost nets in the open ocean (Asmus et al. 2000; Laist 1997; Matsuoka et al. 2005). Most entangled seabirds as well as seals and turtles are reported to be caught in small fragments of trawl net, gillnet, and monofilament line (Laist and Wray 1995).

1.1 Objectives

The general aim of this thesis is to monitor long term changes in plastic ingestion of aquatic birds. The study area of this research is southern Portugal, where Avifauna is abudant due to the geographical position of the Peninsula. Most of the birds can be found in the Ria Formosa lagoon systems, which provide food and shelter. The interest for this region is also given by the facts that open landfills can still be found in this area, it is a touristic place all year round and that it is a recreational fishing area, therefore marine anthropogenic litter is commonly found in the natural environment. The thesis is organized in two papers addressing different objectives in the framework of the general aim. Paper 1 (Chapter 2) assesses the impact and changes of ingested litter over time, focusing on plastic ingestion in four species over a period of seven years (2014 - 2020). Paper 2 (Chapter 3) investigates fishing gear entanglements on 16 bird species over a period of ten years (2010-2019). The specific objectives for each paper are described below:

(1) Describe the temporal trends of anthropogenic litter ingestion in aquatic and marine birds, in southern Portugal. Four bird species with different foraging and migratory behaviours were

selected (*Ciconia ciconia, Larus michahellis, Larus fuscus* and *Morus bassanus*). Data collected in the last seven years (2014-2020) was used. I tested for species-specific differences in the frequency of occurrence, abundance and mass index, type of material ingested as well as recording the colours of the anthropogenic litter. I used the standardised Van Franeker (2011) methodology to allow standardization with other studies and to provide a baseline for future comparison. Changes in time, per species, on the type of plastic ingested, can be useful share light on the origin of anthropogenic litter and design functional management actions.

(2) Describe temporal trends of fishing gear entanglements in 16 aquatic and marine bird species in the south of Portugal, over a period of ten years (2010-2019). For each species the proportions, of individuals affected by entanglement and out of these the proportions of those being lethally affected was calculated. Consequences of entanglements with fishing lines or nets are described.

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CHAPTER 2:
TEMPORAL TRENDS OF PLASTIC INGESTION BY AQUATIC BIRDS IN SOUTHERN PORTUGAL: A MULTISPECIES APPROACH

Silvia Rao[1], Katy R. Nicastro[1,2], María Casero[3], Christopher D. McQuaid[2], Gerardo I. Zardi[2]*

[1]CCMAR-CIMAR – Associated Laboratory, University of Algarve, Campus de Gambelas, Faro 8005–139, Portugal

[2]Department of Zoology and Entomology, Rhodes University, Grahamstown 6140, South Africa

[3]Wildlife Rehabilitation and Investigation Centre (RIAS) – Associação ALDEIA, Ria Formosa Natural Park, Olhão, Portugal

*Corresponding Author: Email: zardi73@yahoo.it

2.1 Abstract

Anthropogenic litter pollution has drastically and exponentially increased in the environment, causing damages in the different ecosystems and impacting wildlife. This has triggered the scientific interest on understanding the full magnitude and impact of litter on coastal and aquatic organisms. Through stomach analysis, the quantitative and qualitative amount of litter can be determined and species-specific changes in litter ingestion can be monitored over time. In this study, we examined stomach contents of four different bird species over a period of seven years (2014-2020). Of the 462 birds analysed, 22.7% ingested anthropogenic litter. All four species examined ingested litter and predominantly plastics. *Ciconia ciconia* had the highest rate in anthropogenic litter (61.1%) and plastic ingestion (55.6%), while *Larus michahellis* had the lowest (13.4% and 1.5% respectively). Despite temporal fluctuations, none of the species showed significant trends throughout the studied period. All species predominantly ingested litter in white/off clear colour. User plastics was the most common category along all species, only Industrial plastics were ingested by *Larus fuscus.*

Keywords: Anthropogenic litter, plastic ingestion, multivariate analysis, aquatic birds, Portugal

2.2 Introduction

Over the last 70 years, anthropogenic litter and in particular that made of plastic has increased exponentially and has rapidly emerged as a global threat to biodiversity (Lau et al. 2020). The rapid and significant accumulation of long-lasting material such as plastics has been particularly pronounced in marine habitats (Haward 2018). Over the last few years, the ubiquitous nature of plastic pollution from coastlines to the open ocean, and from the sea surface to the seafloor has been highlighted (Almroth and Eggert 2019). The negative impacts of plastic and anthropogenic materials in general extend far beyond aesthetic damage. Mounting evidence shows that both the number of taxa affected by litter and the potentially harmful consequences have escalated (Jâms et al. 2020). Anthropogenic litter can lead to physical damages and the toxicity from additives and contaminants absorbed by plastic debris may significantly influence species' behaviour, physiology and survival (Gall and Thompson 2015).

Aquatic birds are especially susceptible to the pervasive and increasing presence of plastic materials in the environment due to their high trophic level and extensive foraging ranges (Avery-Gomm et al. 2012). Some of the earliest indication of plastic pollution in marine organisms were plastic caps, toys and bags ingested by *Laysan albatrosses* in the 1960s (Kenyon and Kridler 1969). Since then, the number of studies stressing the wide range of deleterious effects of plastic pollution on aquatic birds has increased vastly (Battisti et al. 2019). More than 200 avian species, including seabirds, shorebirds, and coastal terrestrial birds are now known to be negatively impacted by anthropogenic waste (e.g. Laist 1997). This number continues to grow as more species are investigated; it is predicted that by 2050, 99% of all seabirds will experience plastic ingestion (Wilcox et al. 2015).

Anthropogenic pollution has a wide range of negative effects on aquatic birds, mainly occurring through ingestion and entanglement (e.g. (Gall and Thompson 2015; J. Provencher et al. 2017; O'Hanlon et al. 2017). Entanglement is mostly passive, when individuals get trapped in lost litter materials, such as fishing nets or plastic bags, or it may be active when individuals became trapped in anthropogenic items that they collect purposely, for example, to construct their nests (Lopes et al. 2020; Ryan 2018). Ingestion can occur inadvertently, while foraging on other prey items, or deliberately when materials resemble natural food items (Wilcox et al. 2015; Cadée 2002). Litter retained within the gut can have direct effects such as dietary dilution causing impaired feeding and growth (Ryan 1988), and physical damage in the form of internal wounds and ulcers and, gastrointestinal obstruction (Puskic et al. 2020). Further, a growing body of evidence shows that birds carrying plastics in their stomach are exposed to potential toxicological effects arising from leaching contaminants that were either added during plastic production or absorbed by the plastics' surface from the surrounding environment (Guo et al. 2020; Roman et al. 2019).

Critically, litter ingested by birds does not necessarily mirror the abundance of plastic waste as well as anthropogenic material in the environment; however, the plastic waste is a reliable proxy for spatial and temporal trends in the abundance and typology of plastic litter in the environment (e.g. Van Franeker et al. 2011a; Van Franeker and Law 2015). For instance, the Northern Fulmar (*Fulmarus glacialis*), a procellariiform seabird distributed across the North Atlantic and Pacific Ocean (Mallory et al. 2006), is used by both OSPAR (Oslo/Paris Convention for the Protection of the Marine Environment of the North-East Atlantic) and the European MSFD (Marine Strategy Framework Directive) for monitoring spatio-temporal fluctuations of plastic waste in the North Sea (OSPAR Commission 2008).

Although the selection of indicator species is crucial to investigate plastic pollution (Avery-Gomm et al. 2012; Mallory, Roberston, and Moenting 2006; Provencher et al. 2009; Van Franeker et al. 2011b), multispecies monitoring (including non-indicator species) is also a key to investigate the pervasiveness of plastic ingestion and to recognize drivers of differences in the quantities and qualities of plastic ingested by different species (Richardson et al. 2013; Bond et al. 2014b; Roman et al. 2016). For instance, surface-feeding seabirds tend to ingest more plastic than pursuit-diving birds because the majority of plastics float and accumulate at the surface (O'Hanlon et al. 2017; Poon et al. 2017a). Over the last few years, several multi-species investigations have been the key to obtain comprehensive information on marine ecosystem health and have highlighted the value of detecting alternative species to use in monitoring programmes (Heidi Acampora et al. 2016). As anthropogenic litter in marine and aquatic habitats continues to rise globally (Trevail et al. 2015), monitoring stomach contents across aquatic species will be increasingly relevant. Importantly, evidence shows that temporal trends of plastic ingestion vary vastly depending on species and plastic typology (Van Franeker et al. 2011b).

The south of Portugal is characterized by several lagoons near the coastline some of which are areas of high diversity of wildlife and key migration stopover and breeding sites for more than a 100 bird species. Only recently, a baseline assessment of the prevalence of plastic litter affecting multi-species of aquatic birds in southern Portugal has been described (Nicastro et al. 2018; Basto et al. 2019). Results shows that the abundance and type of ingested litter varies considerably among species. Such information is fundamental for larger syntheses aimed at assessing changes through time. Here, we used data from these baseline studies in combination with new data to assess temporal changes in litter ingestion by multi-species of aquatic birds in southern Portugal. Specifically, we examined litter pollution ingestion by white storks (*Ciconia ciconia*), lesser black-backed (*Larus fuscus*) and yellow-legged gulls (*Larus michahellis*) and northern gannets (*Morus bassanus*) over a period of 7 years (2014-2020).

2.3 Material and Methods

2.3.1 Study species

The white stork (*Ciconia ciconia*) is globally rated as a less concerned species (IUCN, 2018) and its distribution range spans across Europe, the Middle East, North and South Africa (Cramp and Simmons 1977). In Europe, two populations occur with distinct migratory routes and wintering areas. The occidental population mainly migrates over the Strait of Gibraltar, while the oriental population mainly shifts over the Strait of Bósforo and Israel (A. Araújo et al. 1998). In Portugal, the majority of the population breeds in the south (A. Araújo et al. 1998). A significant decrease in the population was observed between 1950 and 1980 (A. Araújo et al. 1998). The white stork primarily feeds on insects, larvae, amphibian, reptiles, small mammals, annelids and aquatic organisms.

The lesser black-backed gull (*Larus fuscus*) is classified as a less vulnerable species, however, a population reduction over the years was observed (IUCN, 2018). It is a palearctic bird but mostly distributed in the United Kingdom (Hagemeijer et al., 1997). In the Iberian Peninsula, this species is commonly found on the Berlengas Archipelago, on the Pessegueiro Island and in the Ria Formosa coastal lagoon. In Portugal, it nests in the estuarine zones and lagoons, and it is frequently seen on Portugal´s coasts during the winter. The species mainly feeds on insects, fish and human rubbish (“Aves — ICNF”, 2020).

The yellow-legged gull (*Larus michahellis*) is listed as least concern species (IUCN 2018); its distribution goes over the Macaronesia Islands and Northwest Africa through the Mediterranean. It is a migratory species, but some population are defined as partially migratory. The European population is estimated to be high and in expansion in France and the Iberian Peninsula. *Larus michahellis* inhabits coastal as well as inland areas. The yellow-legged gull feeds on fish, insects, molluscs, small mammals and dumb rubbish (“Aves — ICNF”, 2020).

The Northern Gannet (*Morus bassanus*) is a marine bird recorded as a less vulnerable species (IUCN 2018). It is strongly distributed in northern and western Europe, on the east coast of America and Canada and moderate distributed over the Mediterranean area and north-west Africa. During the non-breeding phase, it is extensively dispersed southward. The coast of mainland Portugal is used by this species as feeding ground and wintering area (Ramírez et al. 2008). The Northern Gannet is a piscivore and preys on pelagic fish.

2.3.2 Study area

The lagoon system of Ria Formosa is located in the eastern Algarve and it has been declared Ria Formosa Natural Park (PNRF; Amaral 2010). It comprises a total area of about 18400 ha, of which about 3600 ha are permanently submerged. It covers an extensive area of habitats - marshes, sandbanks and mudflats, dunes, salt flats, lagoons and areas of diverse vegetation. The system is known for its high diversity of Avifauna (Amaral 2009b) mainly made up by species from the orders Gaviiformes, Podicipediformes, Anseriformes, Gruiformes and Charadriiformes, estimating over one hundred species (Farinha and Costa 1999). The wetlands are wintering zones of northern species and the migration route of many birds is over this lagoon.

2.3.3 Procedure

Sampling took place between June 2014 and May 2020 and consisted of selected aquatic and marine birds that had been brought to the wildlife recovery center RIAS, situated in Olhão Portugal, from the region of Algarve and Alentejo. Sampling is based on volunteers and therefore was irregular over time and species. The majority of the animals that reached the center had diseases or traumas. Birds were either dead at their arrival or were euthanised after 24 hours. No animals were euthanised for the benefit of the project. A total of 462 individuals belonging to four species were used for this study: 53 white storks (*C. ciconia*), 154 lesser black-backed (*L. fuscus*) and 196 yellow-legged gulls (*L. michahellis*) and 59 northern gannets (*M. bassanus*). Necropsies were done immediately after death or samples were frozen at - 20°C for later dissections. The necropsies followed the Van Franeker dissection method (Van Franeker 2004). If available, for each individual, data on age (New-Born, Juvenile, Sub-Adult and Adult), gender, body condition and cause of death were registered. Age and gender were determined by the stage of the sexual organs, the body condition (0 to 4) was defined by using pre-determined dimensions (Pinilla 2000). The stomach content was sieved through a 1mm mesh. Inorganic particles were collected and air-dried for two to three days in Petri dishes.

Inorganic particles were counted individually and weighted to the nearest 0.0001 g. (Sartorius advantage AW-224 scale). The items were classified in (a) categories (non-plastic items, industrial plastics or user plastics, the later further classified in sheet-like, fragment, threadlike, foamed, others; Van Franeker et al. 2011;) and, for all plastic recorded, (b) colour (white/clear, grey-silver, black, blue-purple, green, orange-brown, red-pink and yellow; (Provencher et al. 2017).

2.3.4 Data Analysis

Data collected for this study (n = 117) were integrated with data from Nicastro et al. (2018; n = 153) and Basto et al. (2019; n = 192). Samples from these previous studies were from the same region and were treated with the same procedure as described above. The combined dataset consisted of 462 dissected individuals over a period of six and a half years (June 2014 to May 2020). Of the 462 individuals, 105 were affected by litter ingestion (22.73%). For each species and year, percentage frequency of occurrence (% FO) was expressed as the proportion of individuals that ingested litter, plastic and non-plastic items, over the total number of individuals dissected. Additionally, for each individual that ingested litter, abundance (i.e. total number of particles) and total mass were calculated and standardised to the weight of the stomach (hereafter referred as abundance or mass index). When the stomach weight was not available the average weight of the given stomachs of that species was used instead (n=8 samples of *C. ciconia*, average weight 109.46 g; n=11 samples of *M. bassanus*, average weight 37.0 g; n=61 samples of *L. fuscus*, average weight 24.75 g; n=76 samples of *L. michahellis*, average weight 20.78 g). To determine whether there was a correlation between years and either abundance index, mass index or %FO, a Spearman´s rank test was used. Each regression was run with the complete dataset and with a dataset without outliners. In this case the outliners were identified using the Z-score method (Siddharth et al. 2020) in which scores above or below 3, -3 were excluded.

To test for differences in patterns of litter ingestion among years, three data records were used: one with litter abundance for each category, one with litter mass for each category, and one with litter abundance for each colour category. For each data record, a series of one-way multivariate PERMANOVA tests were performed with year of sampling as a fixed factor and litter abundance, mass or colours as the dependent variables. Each multivariate analysis was run with the complete dataset (results reported in supplementary material) and with datasets without outliners. Outliners were identified by assessing the Mahalanobis distance (MD; (Mark and Tunnell 1985). The resulting MD were compared to a chi-square distribution, in which the degrees of freedom were equivalent to the number of predictors. The resulting values were compared against 0.001, data below this value were defined as outliers (Anderson et al. 2008). Each analysis was run with 9999 permutations and using Bray–Curtis dissimilarity matrices for square-root transformed multivariate measures. The Monte Carlo P was preferred over the permutation P (M. J. Anderson 2006). To visually represent each dataset, a two-dimensional non-metric multidimensional scaling (MDS) was plotted.

For the dataset without outliers pairwise tests were performed (Monte Carlo), when the main effect was significant, and permutational analysis of multivariate dispersions test for heterogeneity (PERMDISP) was run to evaluate the variability among years, based on the distances to centroids (Anderson 2006). In addition, the SIMPER procedure (Clarke 1993) was used to identify the percentage contribution (%) that each variable made to the between-years Bray-Curtis dissimilarities and was run with a cut of 50% and only on the dataset without outliers. All multivariate analyses were performed using PRIMER 6.1.15 and PERMANOVA+ 1.0.5 software (PRIMER-E Ltd, 2012).

2.4 Results

Of the total 462 dissected birds, 105 were affected by litter ingestion (22.73%). A total of 551 ingested items were counted with a total mass of 120.2522 g. 58 items were categorized as Non-plastic, mainly glass fragments and four fishing hooks. The most impacted species by anthropogenic litter was *Ciconia ciconia* (61.1%; Figure 1A), 55.6% of all individuals examined ingested plastic items and 20.4% ingested non-plastic litter (Figure 1A). Of all analysed *Morus bassanus,* individuals 22% were affected by litter ingestion, whereas 20.3% were impacted by plastic and 6.8% by non-plastic material (Figure 1B). Of all examined individuals of *Larus fuscus,* 21.4% were affected by anthropogenic litter, more specific 20.8% were affected by plastic litter ingestion and 2.6% by non-plastic (Figure 1C). The evaluated *Larus michahellis* stomachs demonstrated that 13.4% were affected by anthropogenic litter, mainly plastic, since the non-plastic percentage was 1.5% (Figure 1D).

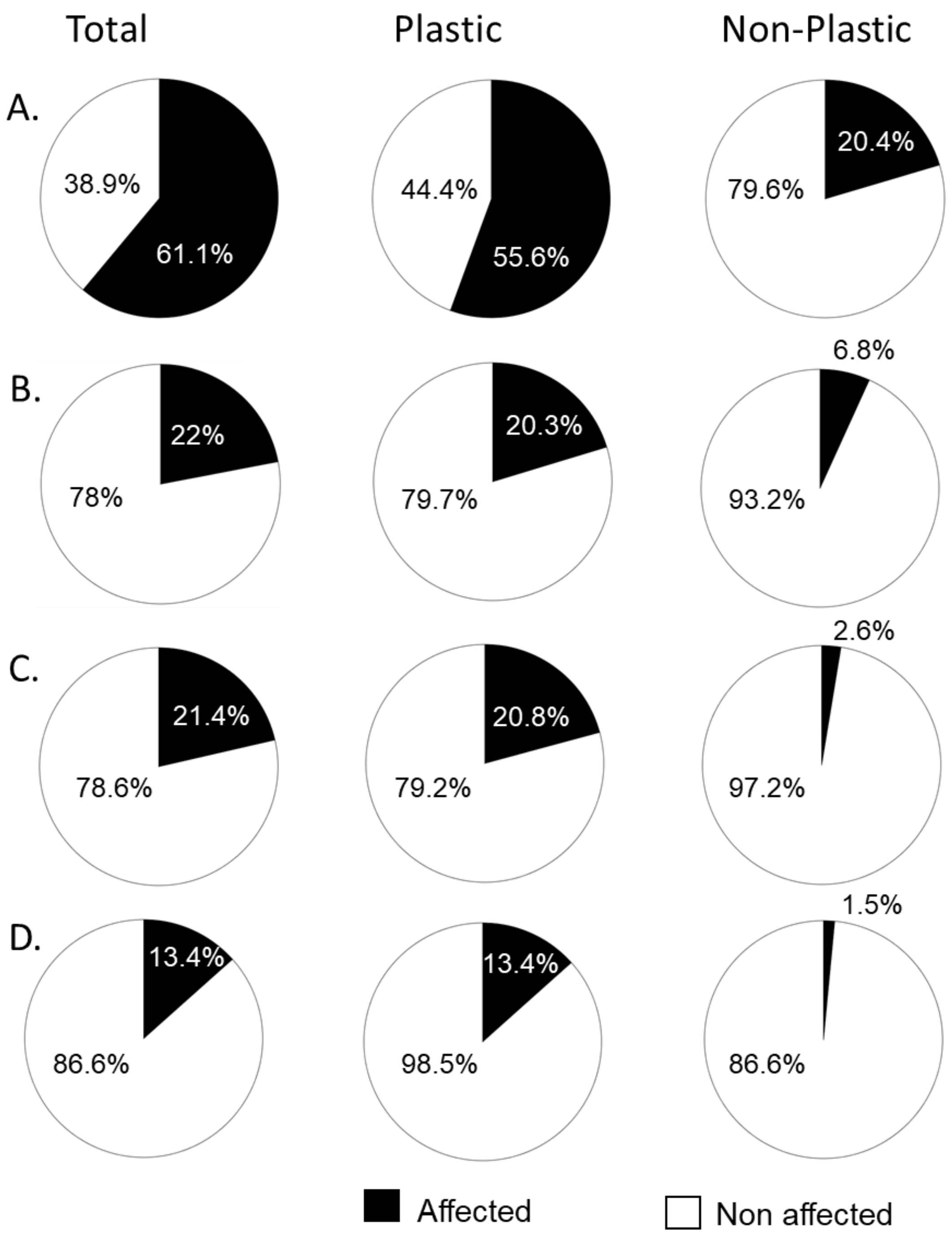

Figure 2.1: Proportion (%) of birds that ingested anthropogenic litter (total plastic and non-plastic items) for (A) *Ciconia ciconia* (n = 52); (B) *Morus bassanus* (n = 56); (C) *Larus fuscus* (n =143); (D) *Larus michahellis* (n = 181).

L. michahellis was the least impacted species (Figure 2.1 and 2.2). The frequency of litter ingestion (%FO) did not significantly increase over the years, nor did the abundance index (Figure 2.3), the mass index increased over time, but not significantly (Figure 2.4). *C. ciconia*, *L. fuscus* and *M. bassanus* had an exponential increase in the litter ingestion (%FO) over the years (Figure 2.22) as well as an increase in the abundance index (Figure 2.3), but none of them were significant. The mass index was not significant for any of the three species, further, a decrease in the mass index over time was observed in *C. ciconia* and *M. bassanus*.

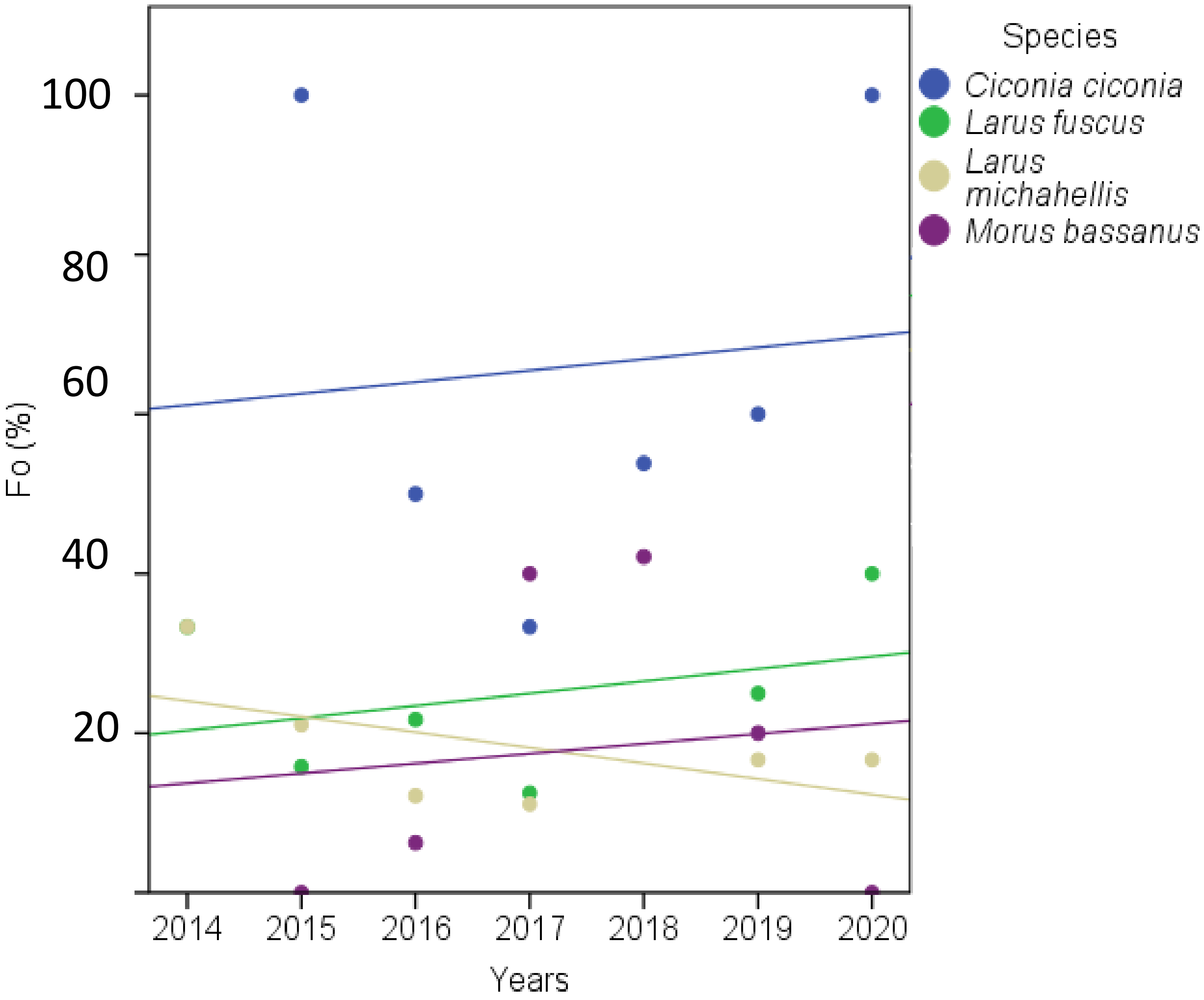

Figure 2.2: Frequency of occurrence (FO%*)* over the years (June 2014 - May 2020) for *Ciconia ciconia*, (P = 0.658, Spearman´s Rho = 0.232), *Larus michahellis* (P = 0.288, Spearman´s Rho = - 0.522), *Larus fuscus (*P = 0.623, Spearman´s Rho = 0.257) and *Morus bassanus* (P = 0.6, Spearman´s Rho = 0.4).

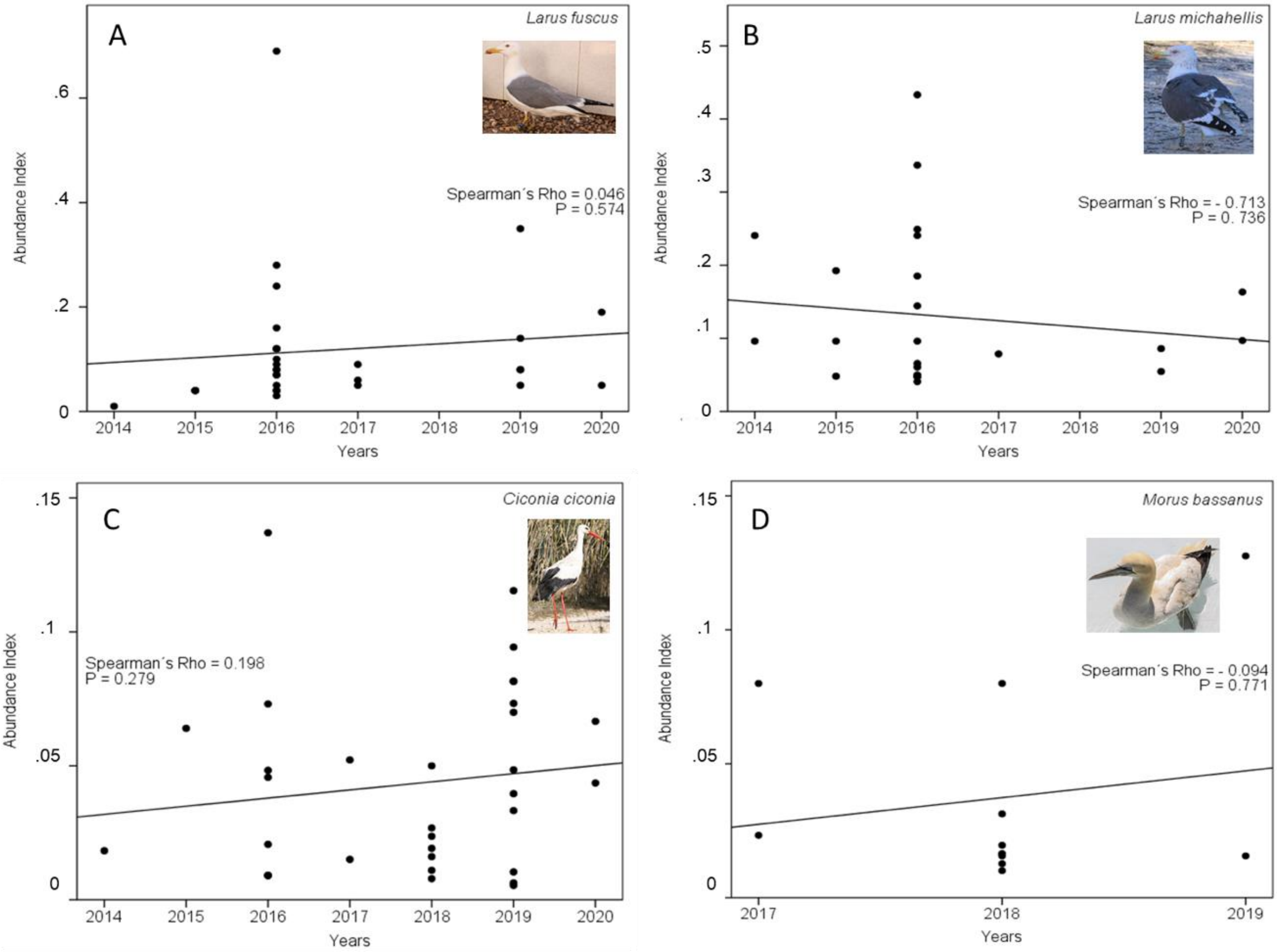

Figure 2.3: Abundance Index of affected birds over the years; (A) *Larus fuscus* (B) *Larus michahellis*, (C) *Ciconia ciconia* and (D) *Morus bassanus*. Spearman´s Rho and Ps for each species are included. Dataset without outliers.

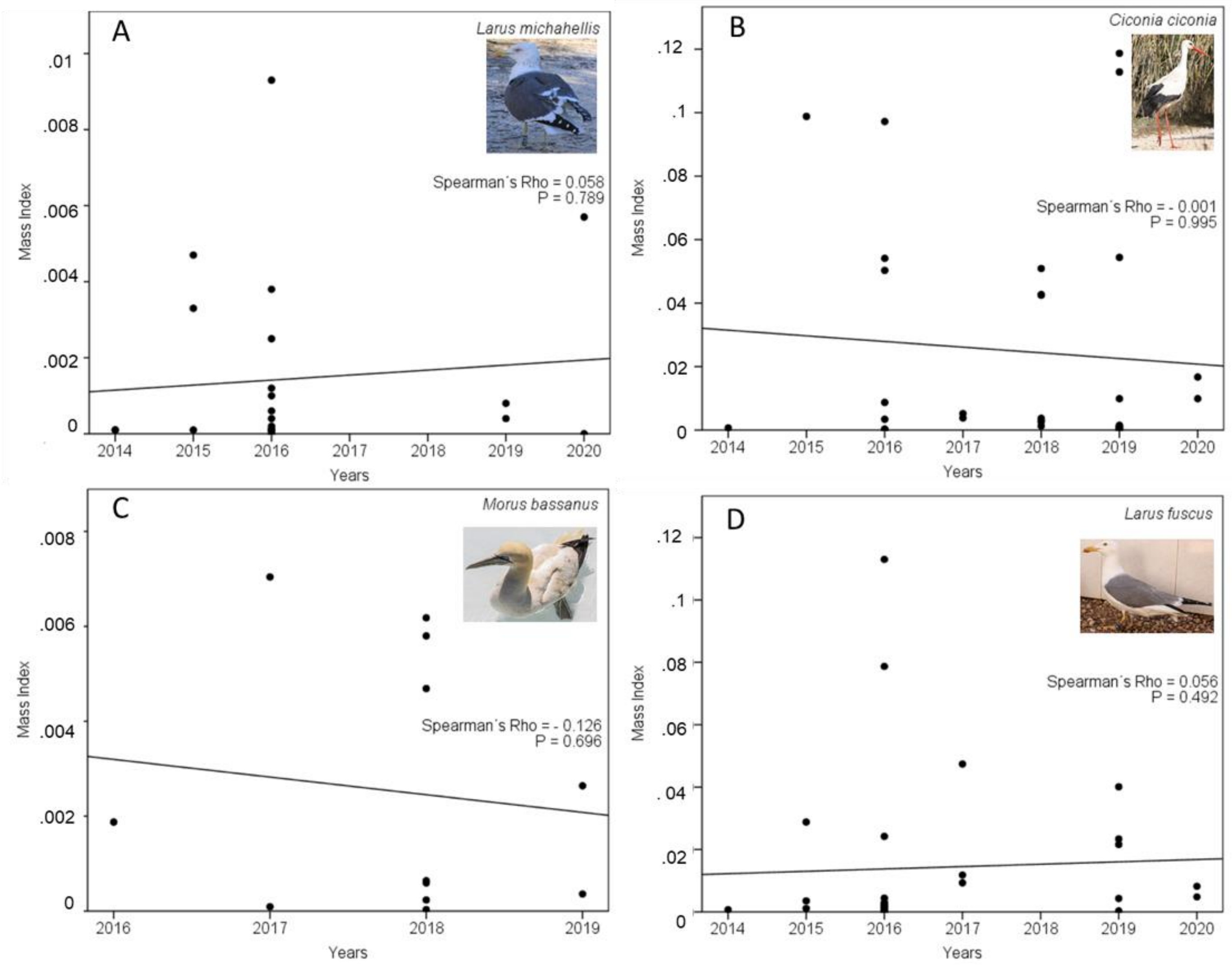

Figure 2.4: Mass index of affected birds over the years; (A) *Larus michahellis*, (B) *Ciconia Ciconia*, (C) *Morus bassanus* and (D) *Larus fuscus*. Spearman´s Rho and P for each species are included. Dataset without outliers.

Results of the PERMANOVA tests showed that years had a significant effect on abundance and mass of litter ingested by *L. fuscus* (without outliers; abundance: $F_{142,137}$ = 2.29, P = 0.04; mass, $F_{143,138}$ = 13.51, P = 0.001; Table S2.1), while colour did not (without outliers; P = 0.0911). However, PERMDISP was also significant for mass (P = 0.0001) and thus the differences observed might be partially explained by the dispersion of the data (Figure 2.5). No significant effects were detected with the full dataset (i.e. including outliers, Fig. S2.3).

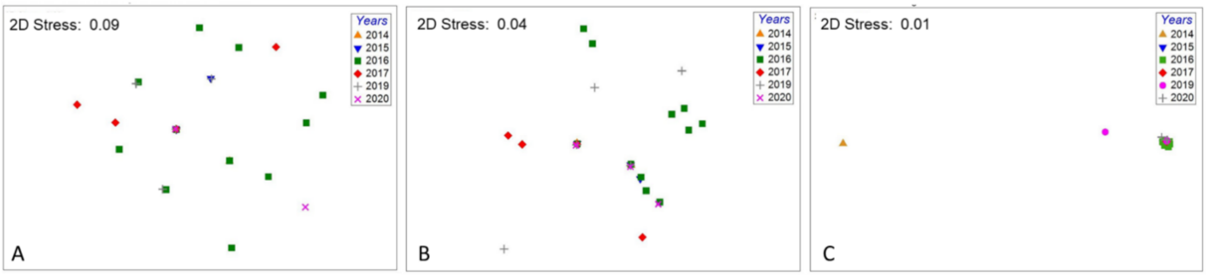

Figure 2.5: *Larus fuscus*; A two-dimensional non-metric multidimensional scaling (MDS) for (A) abundance, (B) colours and (C) mass of the ingested material: Dataset without outliers.

For ingested mass (Figure S2.7), Sheetlike contributed the most to the differences observed between 2014 and 2015 (pairwise test: P (MC) = 0.0518; SIMPER: 66.67%). Between 2016 and 2020, Others (pairwise test: P (MC) = 0.0244; SIMPER: 38.86%) and Non-plastic (pairwise test: P (MC) = 0.0244; SIMPER: 37.27%) contributed the most to the difference observed. Between 2017 and 2020, Others provided the highest contribution to the observed differences (pairwise test: P (MC) = 0.0299; SIMPER: 50.00%). Between 2015 and 2020 Non-plastic (pairwise test: P (MC) = 0.0202; SIMPER: 37.50%) and Others (pairwise test: P (MC) = 0.0202; SIMPER: 34.74%) contributed the most to the differences observed. Between 2019 and 2020, Non-plastic contributed the most to the difference observed (pairwise test: P (MC) = 0.0517; SIMPER: 51.67%).

Years had a significant effect on litter mass ingested by *L. michahellis* with (PERMANOVA; $F_{193,188}$ = 5.4062, P = 0.0001; Table S2.1) and without outliers ($F_{142,137}$ = = 13.79, P = 0.0001; Table S1). For ingested mass (Figure S2.8), Fragments contributed the most to the differences observed between 2014 and 2016 (pairwise test: P (MC) = 0.0001; SIMPER: 92.28%), 2014 and 2017 (pairwise test: P (MC) = 0.0003; SIMPER: 100.00%), 2014 and 2019 (pairwise test: P (MC) = 0.001; SIMPER: 73.48%) and 2014 and 2020 (pairwise test: P (MC) = 0.0001; SIMPER: 100.00%). Between 2015 and 2019 Threadlike (pairwise test: P (MC) = 0.041; SIMPER: 28.67%) and Sheetlike (pairwise test: P (MC) = 0.041; SIMPER: 25.27%) contributed the most to the difference observed. Sheetlike contributed the most to the difference observed between 2015 and 2016 (pairwise test: P (MC) = 0.0001; SIMPER: 57.17%), 2015 and 2020 (pairwise test: P (MC) = 0.0134; SIMPER: 61.26%), 2016 and 2017 (pairwise test: P (MC) = 0.001; SIMPER: 51.79%) and 2016 and 2020 (pairwise test: P (MC) = 0.0001; SIMPER: 51.79%).

PERMDISP for mass was only significant when testing the dataset with outliers (PERMDISP P = 0.0399). Years had a significant effect on the litter colour in the data with outliers with no significant difference in the dispersion of the data ($F_{193,188}$ = 2.3908, P = 0.0454; Table S2.1, PERMDISP P = 0.4417). No effect was detected for abundance (P = 0.0922; Table S2.1).

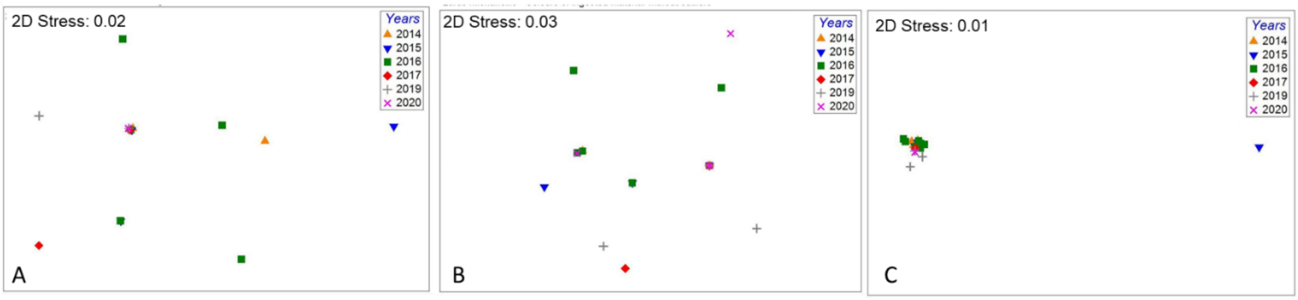

Figure 2.6: Larus michahellis; A two-dimensional non-metric multidimensional scaling (MDS) for (A) abundance, (B) colours and (C) mass of the ingested material: Dataset without outliers.

The amount of litter ingested by *C. ciconia* changed over the years in terms of abundance, both in the dataset with ($F_{53,43}$ = 1.997, P = 0.0494; Table S1) and without outliers ($F_{51,45}$ = 2.2339, P = 0.02). This was not the case for mass (P = 0.2558; Table S2.1) or colour (P = 0.1234; Table S1). For ingested abundance (Figure S2.9), between 2015 and 2017, Others (pairwise test: P (MC) = 0.0338; SIMPER: 40.58%) and Fragments (pairwise test: P (MC) = 0.0338; SIMPER: 34.81%) contributed the most to the difference observed. Between 2015 and 2018 Others and Fragments also provided the highest contribution to the observed differences (Other: pairwise test: P (MC) = 0.0153; SIMPER: 40.48%; Fragments: pairwise test: P (MC) = 0.0153; SIMPER: 29.49%). Threadlike contributed the most to the difference observed between 2016 and 2020 (pairwise test: P (MC) = 0.0225; SIMPER: 45.49%), 2017 and 2020 (pairwise test: P (MC) = 0.0344; SIMPER: 53.72%) and 2018 and 2020 (pairwise test: P (MC) = 0.0217; SIMPER: 51.83%). No significant differences in the dispersion of the data were detected for abundance (PERMDISP P = 0.1718 and P = 0.2953 for the full dataset and the dataset without outliers respectively).

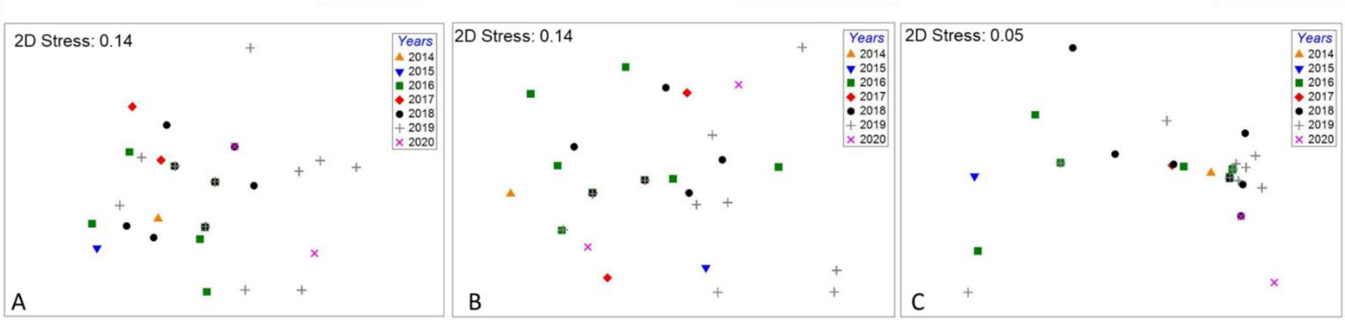

Figure 2.7: *Ciconia ciconia;* A two-dimensional non-metric multidimensional scaling (MDS) for (A) abundance, (B) colours and (C) mass of the ingested material; Dataset without outliers.

No changes in abundance (P = 0.2963), mass (P = 0.6389) or colour (P = 0.6734) were observed over the years for *M. bassanus* (Table S2.1).

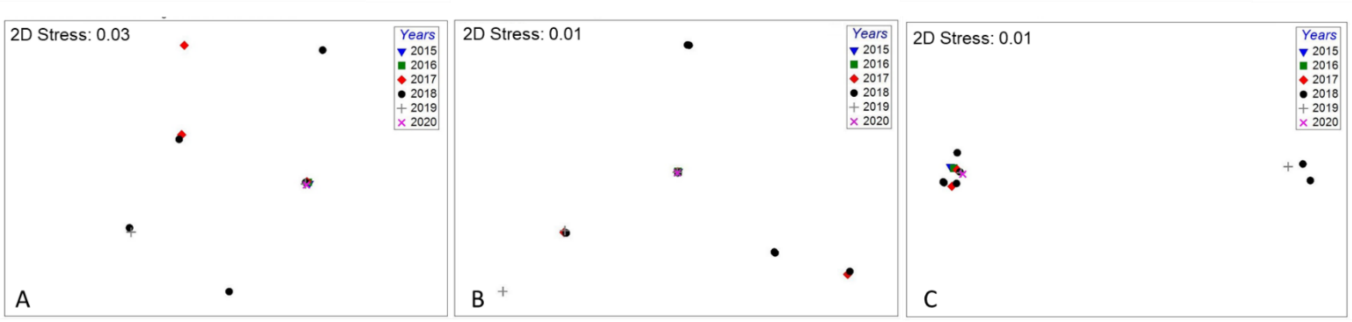

Figure 2.8: *Morus bassanus;* A two-dimensional non-metric multidimensional scaling (MDS) for (A) abundance, (B) colours and (C) mass of the ingested material; Dataset without outliers.

2.5 Discussion

Overall, it was shown that all four species were affected by anthropogenic litter ingestion with plastic as the dominant material. Interestingly, despite marked temporal fluctuations in the percentage of individuals with litters inside their stomachs, none of the species showed significant trends throughout the study period. Similarly, despite the abundance and the mass of some specific ingested litter categories changes significantly between years, no significant increasing or decreasing trends were observed.

A temporal study on Fulmars in the Canadian Arctic reported an increment in plastic litter entering Arctic waters correlated with the increasing ingestion of plastics by Arctic seabirds over time. Therefore, they identified this species as an appropriate species to track the incidence of marine litter (Provencher 2009). To better understand the use of anthropogenic material by *Larus michahellis* in the construction of breeding nests in Portugal, a temporal examination of nests was done, highlighting that nests in urban areas were highly affected compared to nests in natural breeding sites (Lopes et al. 2020). In a temporal multispecies baseline for incidence of plastic ingestion, Basto et al. (2019) found evidence of the most common type of plastic and most common colour ingested by six bird species, giving a main basis for the understanding of their foraging behaviour. In Scotland, between 1969 and 2007 a low but steady frequency of occurrence of plastic ingestion of seabirds was observed (Harris and Wanless 1994; Harris and Wanless 2011), whereas in a temporal study over 27 years no significant change was found in the proportions of deep- water seabirds that ingested plastic, implying that these species ingest plastic at constant ratio regardless of the increasing pollution (Bond et al. 2013).

Despite non-significant temporal tendencies, we observed pronounced interspecies differences in plastic ingestion, confirming previous studies showing that the propensity of a species to ingest plastic often varies according to foraging behaviour, foraging range, morphology or diet (Richardson et al. 2013; Moser and Lee 1992; Poon et al. 2017; Bond et al. 2014a).

It has been suggested that, as plastics occur at higher densities in offshore waters seabirds that feed farther offshore may be more vulnerable to plastic ingestion than inshore species (Richardson et al. 2013). Contrary to this expectation, in our study, *C. ciconia*, which generally forage inland or in coastal shallow waters, showed consistently higher frequencies of occurrence compared to the other three species. Indeed, there is mounting evidence that Ciconiidae are becoming increasingly dependent on terrestrial anthropogenic food resources. Although our data do not allow to establish if the relatively larger mass and abundance of plastic items found in stomachs of storks originated from human-related environments, numerous recent studies have described the growing use of agricultural areas and landfill sites by

European white storks (N. I. Gilbert et al. 2016). For instance, in Spain, rubbish dumps are the major food source for storks, contributing nearly 70% of their diets and a year-round availability of foraging resources on landfill, which compensates for seasonal declines in natural food availability (Tortosa et al. 2002).

Exploitation of landfill sites has marked behaviour and fitness (N. I. Gilbert et al. 2016; Peris 2003). For instance, breeding success has improved mainly due to a reduced mortality in first-year birds (Gilbert et al. 2016). Similarly, in northern Algeria, *C. ciconia* breeding colonies exploiting landfill sites display increases in egg volume and hatching mass (Djerdali et al. 2016). The continuous and abundant food resources from rubbish dumps also affected white storks' home ranges and migratory patterns eventually enabling the establishment of resident individuals in a formerly wholly migratory species (Blanco 1996; Tortosa et al. 2002). Most importantly, significant population demography changes have also been observed; over the last twenty years, the breeding population of the white stork has increased significantly in Iberia, with the number of overwintering white storks rising by an order of magnitude (Rosa et al. 2009).

In addition to feeding strategies, for how long ingested litter is retained in the bird's digestive tract may be responsible for large interspecific differences. For instance, gull species regurgitate large amounts of the debris ingested, thus the assessment of stomach contents is only a snapshot of ingestion. Thus, while they may still be exposed to chemical contaminants from ingested litter, retention times are likely to be comparatively shorter than those of storks, explaining the interspecific differences observed in this study.

Importantly, retention time of plastics is also largely influenced by numerous other factors such as the size or shape of an ingested item, material/polymer type, and the presence of previously ingested natural items. Retention times of plastic ingestion in marine wildlife remain largely unknown, however, evidence shows that microplastics pass through the intestines of marine megafauna relatively quickly, with significantly shorter retention times compared to larger pieces, that must be broken down before being expelled. Moreover, previous studies have shown that large and softer plastic items such as latex balloon fragments, bags and foam can reside in the gut of marine wildlife for several months. Results reported here confirm previous findings by showing that *C. ciconia* by highlighting a remarkably high occurrence of silicones, mostly in the form of warm coloured rubber bands and elastics. It has been suggested that storks are particularly exposed to such items because their colour, shape and softness mimic Lumbricidae, a main component of their diet (e.g., Sazima and D'angelo 2015).

The propensity for worm-like debris in *C. ciconia*, together with different feeding ranges and retention times, lead to the overall higher mass of ingested litter compared to the other species studied here.

The ingestions of User plastics were substantially prevailing over the other materials ingested. The exposure is given by the facilitated entrance of these materials into the ecosystems directly from landfills (UNEP et al. 2016), and so far, one-third of plastic litter produced in Portugal gets into open landfills (PlasticsEurope 2018), which consequently is a direct food resource for aquatic birds.

Older studies assessed that seabirds were not severely affected by plastic ingestion (Furness 1985; Ryan 1987; Ryan and Jackson 1987; Moser and Lee 1992), however an increase in seabird species and a growth in the frequency of occurrence was observed over time (Robards et al. 1995; Wilcox et al. 2015). The mortality rate in seabirds is difficult to be associated with plastic ingestion, because most of the animals suffer from obstruction and die indirectly due to starvation (Pierce et al. 2004). The northern gannet is found in diverse recent examinations, were the outcomes exemplify the results of this study with relatively high frequencies of plastic ingestion, as reported in a study conducted in Ireland by Acampora et al. (2016) where 32% were affected. Codina-García et al. (2013) reported an intermediate incidence of plastic ingestion (13%) in *Morus bassanus* species examined in the Mediterranean Sea. Current studies effected in southern Portugal, as this examination, showed relatively low frequency of occurrence or none (Basto et al. 2019; Nicastro et al. 2018), anyhow the samples of these two examinations were integrated into this study, which demonstrated the exposure of *M. bassanus* to plastic ingestion, indicating the importance of increasing temporal analysis.

Our research shows a solid record of the existence of anthropogenic litter in the diet of the examined species and the results suggest that the ingested materials might be found in the enclosing foraging areas (Ryan and Moloney 1990). Multispecies surveys to analyse plastic ingestion have been increasing in the last years and will further rise, given that new species and regions are increasingly affected by marine plastic pollution.

Forthcoming research should compare anthropogenic material ingested by birds with the surrounding (Spear et al. 1995; Acampora et al.2015), and engage in long-term monitoring of plastic ingestion by aquatic birds, applying standardized methods (Van Franeker et al. 2011a), because only by adopting this strategy, data can be compared crosswise spatial and temporal range.

We are facing a substantial change in the environment due to anthropogenic actions, of which plastic pollution and anthropogenic litter have been described as major threats. Thus, this study provides a crucial initial temporal assessment aimed at understanding how increases in this form of pollution affects wildlife overtime.

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2.6 Supplementary material

2.6.1 Tables

Table S2.1: P values for each species for the three categories: Abundance, Mass and Colour, with and without outliers.

	Abundance P-value	**Mass P-value**	**Colour P-value**
Larus fuscus	0.4494	0.1189	0.2266
Larus fuscus data without outliers	0.0464*	0.001**	0.0911
Larus michahellis	0.0304*	0.0197**	0.0454*
Larus michahellis data without outliers	0.0922	0.0001*	0.1628
Ciconia ciconia	0.0494*	0.2558	0.1234
Ciconia ciconia data without outliers	0.0209*	0.0752	0.1628
Morus bassanus	0.2963	0.6389	0.6734
Morus bassanus data without outliers	0.1859	0.2608	0.6398

* significant P; **significant P and PERMDISP

2.6.2 Figures

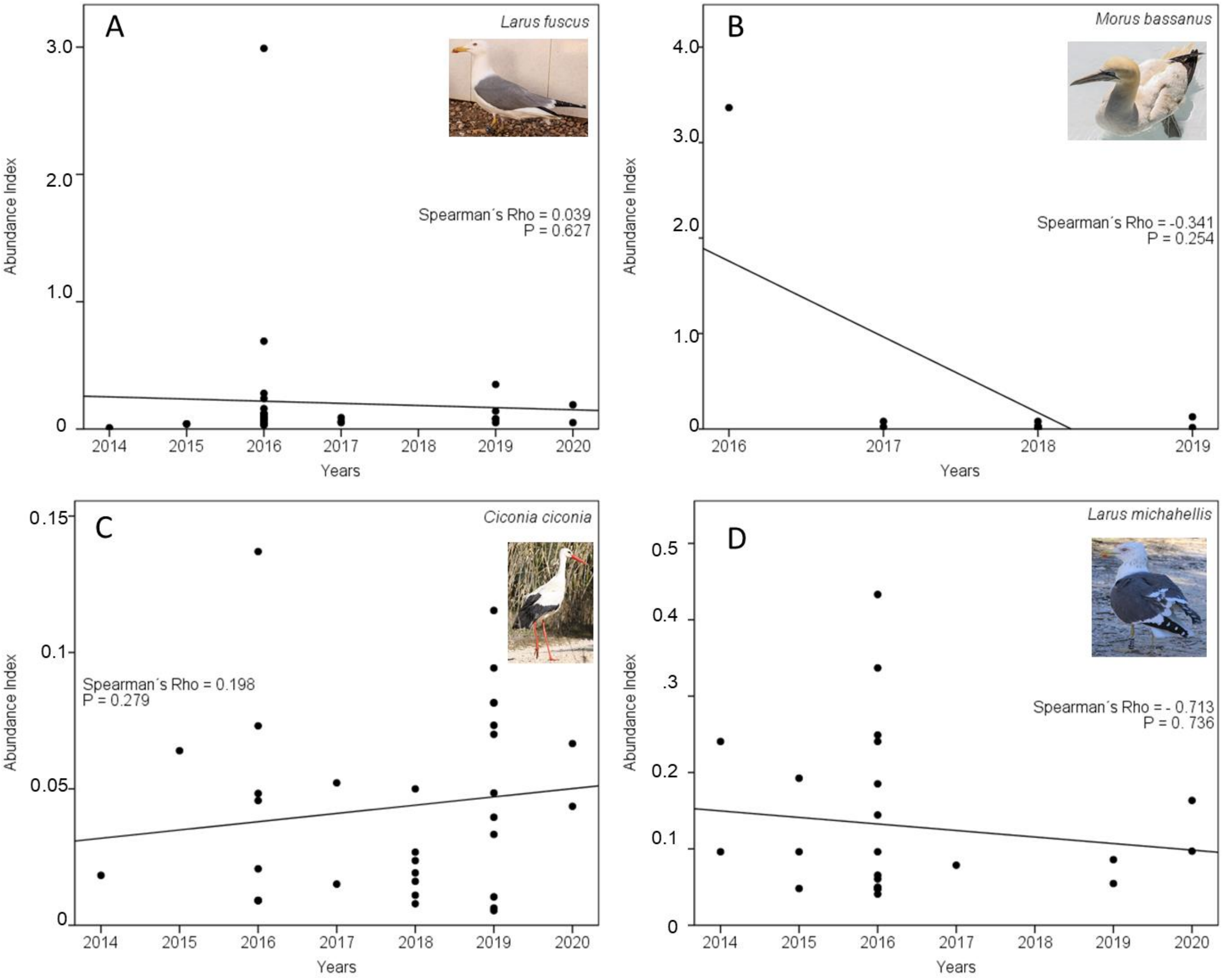

Figure S2.1: Abundance Index of affected birds over the years; (A) *Larus fuscus*, (B) *Morus bassanus*, (C) *Ciconia ciconia* and (D) *Larus michahellis*. Including Spearman´s Rho and P for each species.

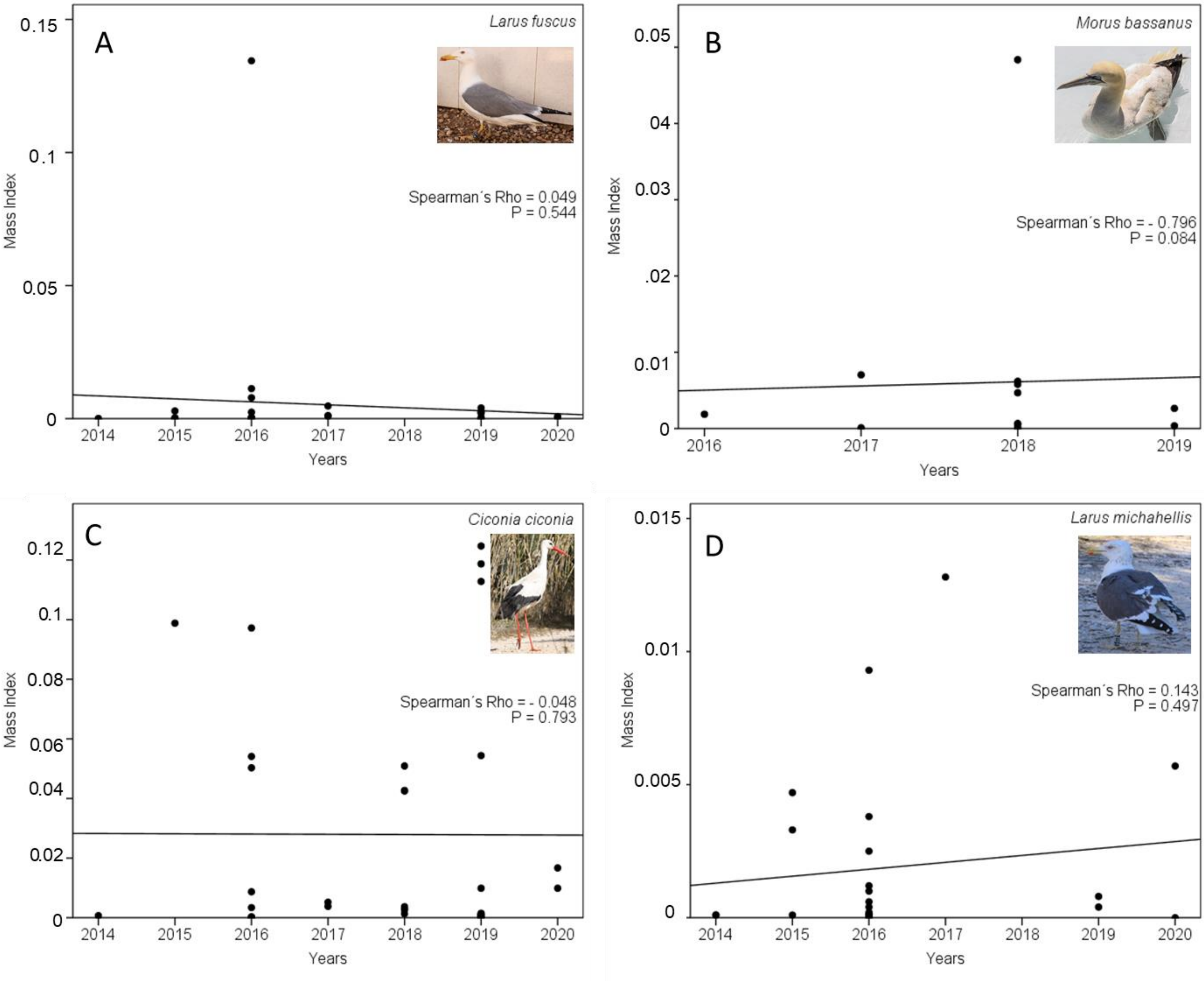

Figure S2.2: Mass index of affected birds over the years; (A*) Larus fuscus*, (B) *Morus bassanus,* (C) *Ciconia ciconia* and (D) *Larus michahellis*. Including Spearman´s Rho and P for each species.

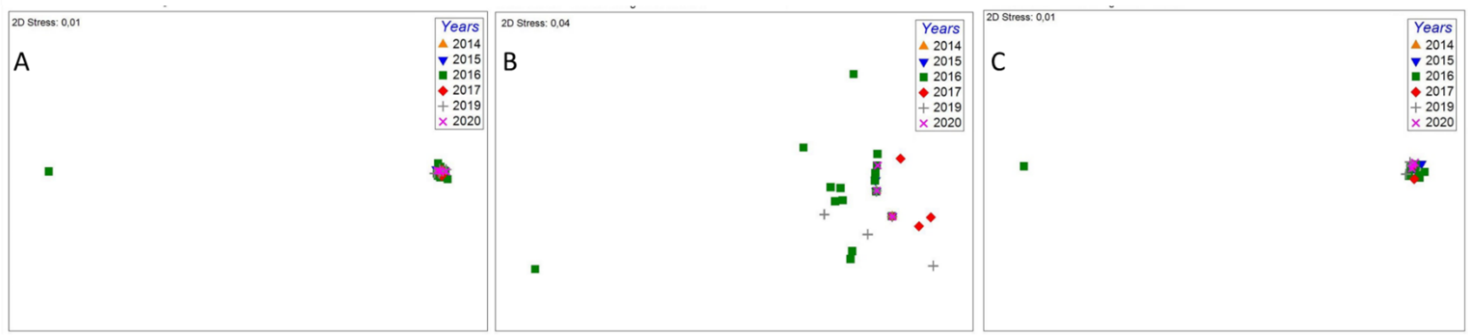

Figure S2.3: *Larus fuscus;* A two-dimensional non-metric multidimensional scaling (MDS) for (A) abundance, (B) colours and (C) mass of the ingested material: Dataset with outliers.

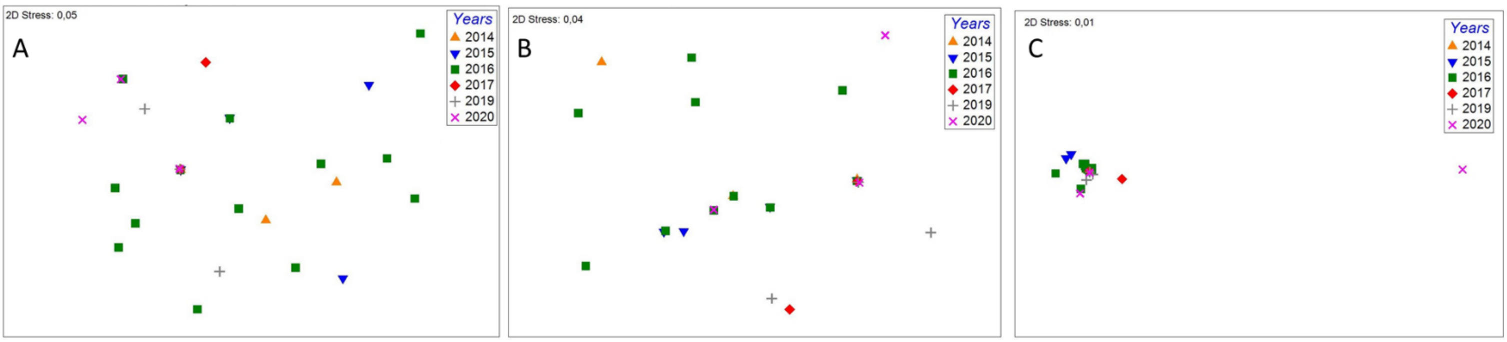

Figure S2.4: *Larus michahellis;* A two-dimensional non-metric multidimensional scaling (MDS) for (A) abundance, (B) colours and (C) mass of the ingested material: Dataset with outliers.

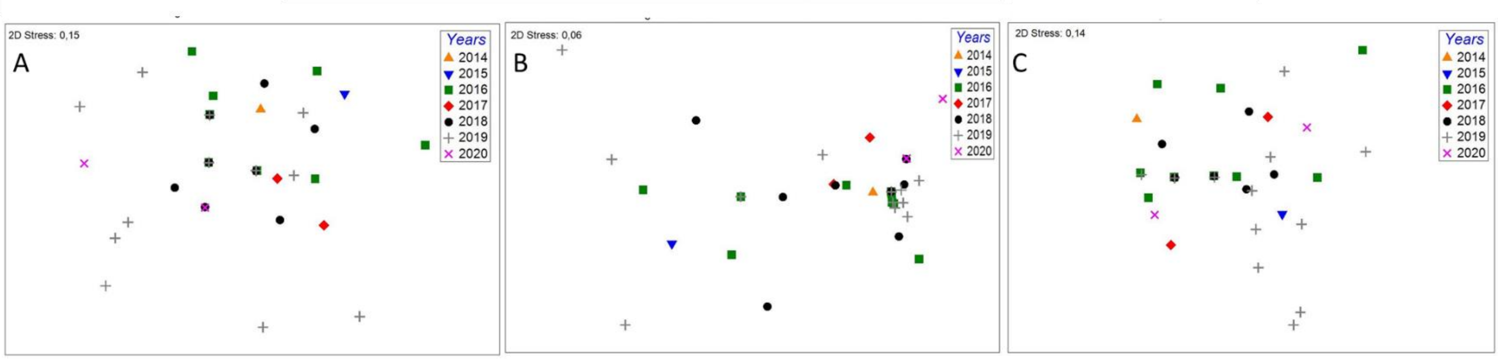

Figure S2.5: *Ciconia ciconia;* A two-dimensional non-metric multidimensional scaling (MDS) for (A) abundance, (B) colours and (C) mass of the ingested material: Dataset with outliers.

Figure S2.6*: Morus bassanus;* A two-dimensional non-metric multidimensional scaling (MDS) for (A) abundance, (B) colours and (C) mass of the ingested material: Dataset with outliers.

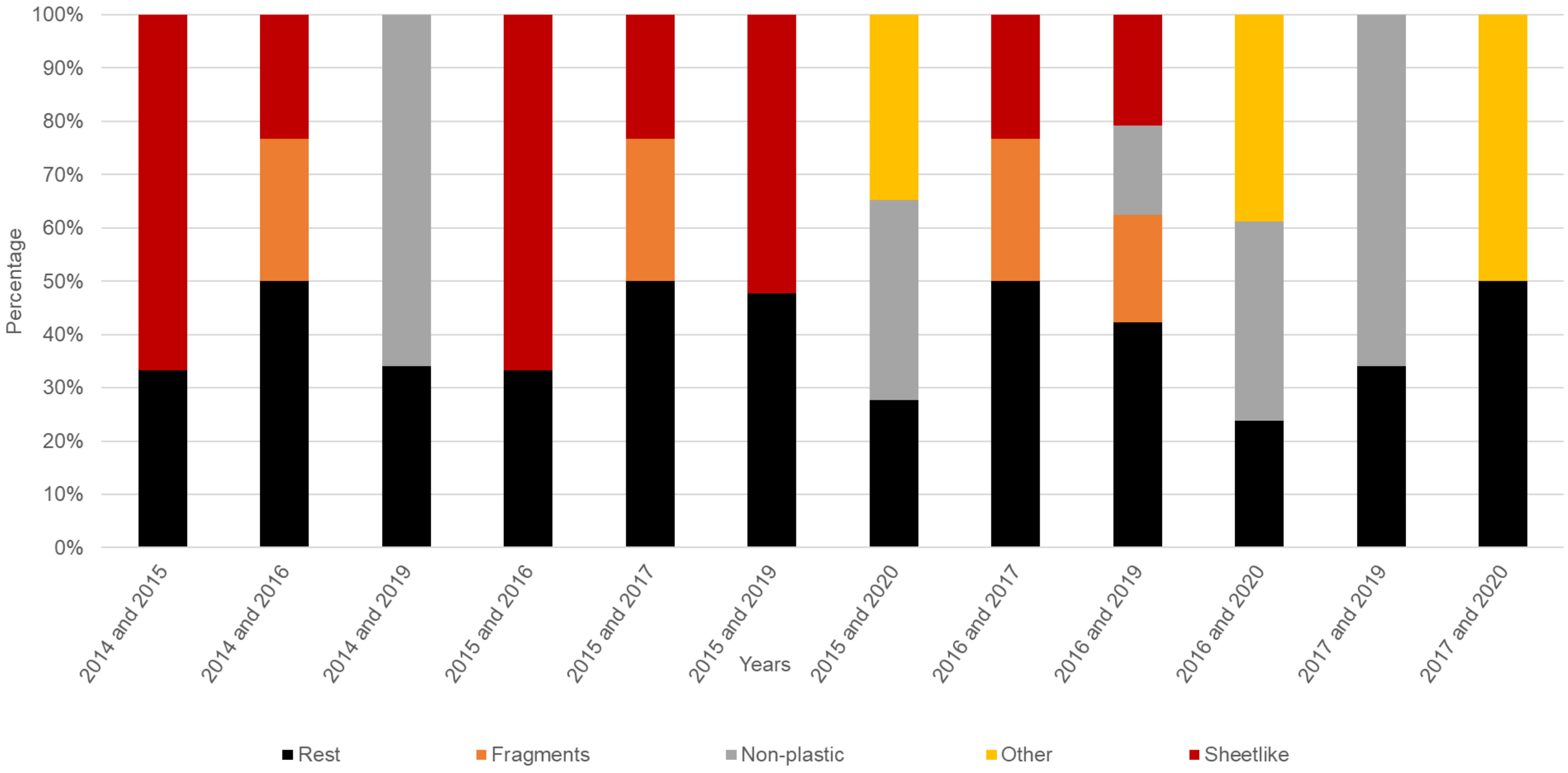

Figure S2.7: *Larus fuscus*; Pair-wise testing and Simper in a visual representation. Mass of ingested material divided into categories between the years 2014 and 2020.

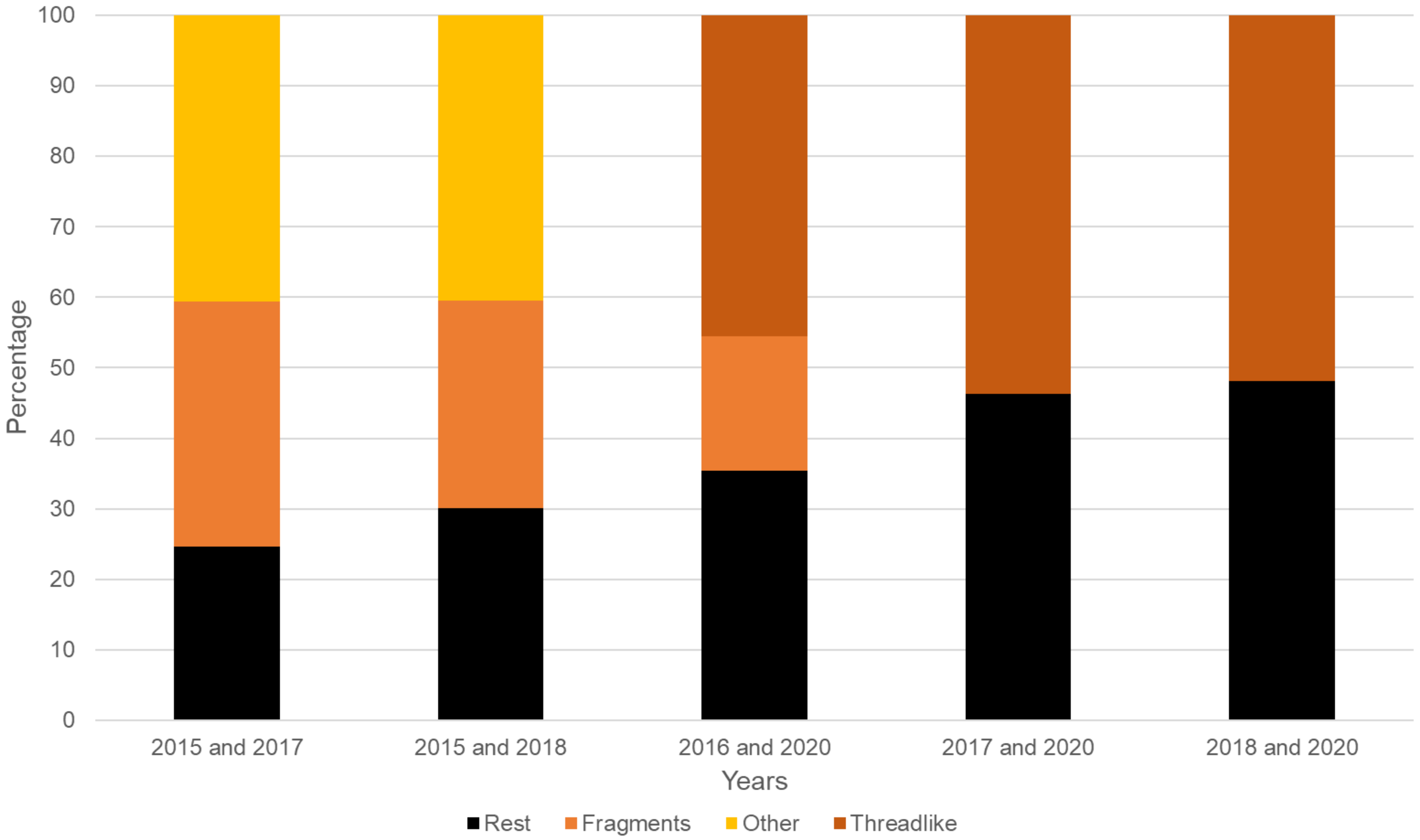

Figure S2.8: *Larus michahellis*; Pair-wise testing and Simper in a visual representation. Mass of ingested material divided into categories between the years 2014 and 2020.

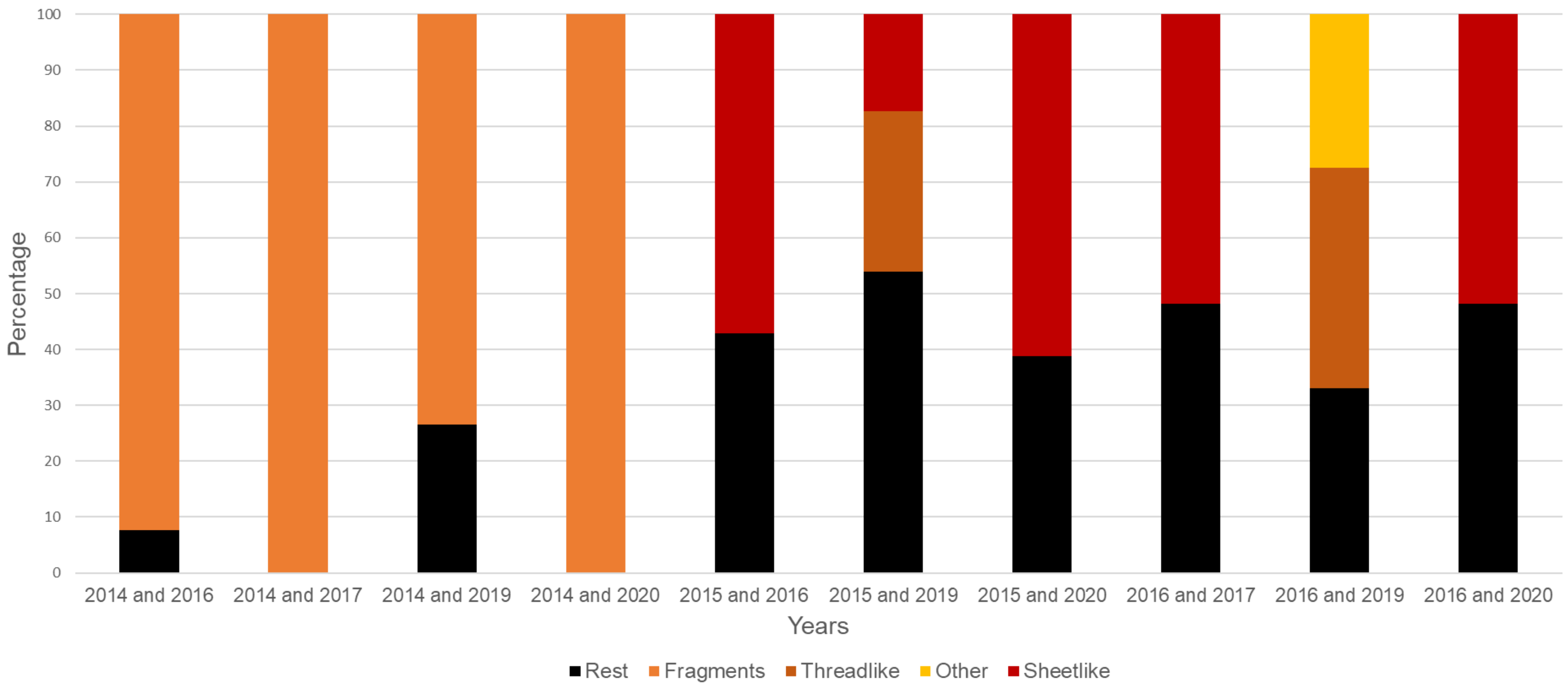

Figure S2.9: *Ciconia ciconia*; Pair-wise testing and Simper in a visual representation. Abundance of ingested material divided into categories between the years 2014 and 2020.

Table S2.2: Data on the plastics ingested by *Ciconia ciconia* (n=110) based on plastic categories. Frequency of occurrence of plastics (95% confidence intervals – CI) and plastic litter abundance. Abundance was calculated using affected individuals.

	Frequency of plastic occurrence (%FO)	Number of plastic items				Mass of plastic items		
	(95% CI)	Mean (n; ± sd; ± se)	Median	Range		Mean (g; ± sd; ± se)	Median	Range
Global	44.54 (1.839, 2.561)	2.2 (110; ± 1.931; ± 0.276)	2	1-12		2.1 (102.847; ± 3.84; ± 0.549)	0.29	0.0008-18.16
Industrial		0	0	0		0	0	0
User	29.1 (1.434, 1.966)	1.7 (54; ± 0.998; ± 0.176)	1	1-4		0.462 (14.769; ± 1.423; ± 0.251)	0.04	0.0008-8.0738
Sheetlike	8.18 (1.232, 2.968)	2.1 (19; ± 1.189; ± 0.217)	0	1-4		0.055 (0.491; ± 0.055; ± 0.01)	0	0.0022-0.2885
Threadlike	5.45 (0.238, 2.762)	1.5 (9; ± 0.712; ± 0.132)	0	1-3		1.828 (10.965; ± 1.53; ± 0.289)	0	0.2458-8.0738
Foam		0	0	0		0	0	0
Fragments	15.45 (0.758, 2.242)	1.5 (26; ± 0.9979; ± 0.176)	1	1-4		0.195 (3.312; ± 0.23; ± 0.04)	0	0.0008-0.9474
Other	15.45 (2.609, 3.971)	3.29 (56; ± 2.6; ± 0.504)	0	1-12		5.181 (88.079; ± 4.669; ± 0.899)	0.63	0.0723-18.16

Table S2.3: Data on the plastics ingested by *Morus bassanus* (n=137) based on plastic categories. Frequency of occurrence of plastics (95% confidence intervals – CI) and plastic litter abundance. Abundance was calculated using affected individuals.

	Frequency of plastic occurrence (%FO)	Number of plastic items				Mass of plastic items		
	(95% CI)	Mean (n; ± sd; ± se)	Median	Range		Mean (g; ± sd; ± se)	Median	Range
Global	9.49 5.39, 16.61)	11 (137; ± 33.495; ± 9.29)	1	1-122		0.413 (4.125; ± 0.981; ± 0.193)	0.012	0.002-3.82
Industrial		0	0	0		0	0	0
User	7.3 (6.51, 19.49)	13 (133; ± 38.198; ± 12.079)	1	1-122		0.57 (3.989; ± 1.342; ± 0.448)	00.01	0.0017-3.82
Sheetlike		0	0	0		0	0	0
Threadlike	5.84 (-22.21, 24.21)	1 (8; ± 0.467; ± 0.14)	1	1-1		0.783 (3.913; ± 0.899; ± 0.212)	0	0.0017-3.8201
Foam	0.73	122	0	0		0.0085	0	0
Fragments	0.73	3	0	0		0.0676	0	0
Other	2.19 (-31.82, 33.82)	1 (4; ± 0.816; ± 0.333)	0	1-2		0.045 (0.136; ± 0.899; ± 0.212)	0	0.0157-0.1011

Table S2.4: Data on the plastics ingested by *Larus fuscus* (n=175) based on plastic categories. Frequency of occurrence of plastics (95% confidence intervals – CI) and plastic litter abundance. Abundance was calculated using affected individuals.

	Frequency of plastic occurrence (%FO)	Number of plastic items				Mass of plastic items		
		Mean	Median	Range		Mean	Median	Range
	(95% CI)	(n; ± sd; ± se)				(g; ± sd; ± se)		
Global	24.57	4	1	1-90		0.118	0	0.0001-4.052
	(2.01, 5.99)	(175; ± 13.454; ± 1.987)				(4.849; ± 0.631; ± 0.05)		
Industrial	1.71	31	2	1-90		1.37	0	0.018-4.052
	(20.61, 41.39)	(93; ± 51.1; ± 29.5)				(4.111; ± 0.954; ± 0.225)		
User	18.86	2	1	1-14		0.019	0	0.0001-0.2793
	(1.45, 2.55)	(70; ± 2.333; ± 0.386)				(0.593; ± 0.052; ± 0.004)		
Sheetlike	6.86	1.5	0	1-3		0.018	0	0.0004-0.1054
	(-4.715, 7.715)	(18; ± 0.87; ± 0.164)				(0.195; ± 0.009; ± 0.001)		
Threadlike	4	1.57	0	1-3		0.004	0	0.0001-0.0111
	(-6.3807, 9.5207	(11; ± 0.82; ± 0.152)				(0.03; ± 2.646; ± 0.461)		
Foam	2.29	2	0	1-5		0.003	0	0.0012-0.0077
	(-7.32, 11.32)	(8; ± 1.342; ± 0.359)				(0.014; ± 2.646; ± 0.461)		
Fragments	5.71	3.3	0	1-14		0.039	0	0.0027-0.2793
	(2.397, 4.203)	(33; ± 2.646; ± 0.461)				(0.353; ± 0.023; ± 0.002)		
Other	4	1.86	0	1-6		0.02	0	0.0004-0.0527
	(-5.752,9.4722)	(12; ± 1.552; ± 0.461)				(0.145; ± 0.009; ± 0.001)		

Table S2.5: Data on the plastics ingested by *Larus michahellis* (n=70) based on plastic categories. Frequency of occurrence of plastics (95% confidence intervals – CI) and plastic litter abundance. Abundance was calculated using affected individuals.

	Frequency of plastic occurrence (%FO)	Number of plastic items				Mass of plastic items		
	(95% CI)	Mean (n; ± sd; ± se)	Median	Range		Mean (g; ± sd; ± se)	Median	Range
Global	44.29 (1.5644, 2.2156	1,89 (70; ± 1.3901; ± 0.1265)	1	1-6		0.026 (0.7411; ± 0.456 ; ± 0.004)	0	0.0001-0.163
Industrial	0	0	0	0		0	0	0
User	40.00 (1.6288, 2.3112)	1.97 (67; ± 1.425; ± 0.131)	1	1-6		0.02 (0.529; ± 0.575; ± 0.041)	0	0.0001-0.163
Sheetlike	11.43 (1.37, 2.63)	2 (19; ± 1.227; ± 0.289)	0	1-5		0.021 (0.186; ± 0.013; ± 0.001)	0	0.0001-0.163
Threadlike	8.57 (1.14, 2.86)	2 (10; ± 0.984; ± 0.232)	0	1-3		0.0436 (0.174; ± 0.796; ± 0.04)	0.0048	0.0016-0.163
Foam	4.29 (0.78, 3.22)	2 (5; ± 0.951; ± 0.36)	0	1-2		0.0037 (0.0074; ± 0.002; ± 0.001)	0	0.0029-0.0045
Fragments	15.71 (1.826, 2.894)	2.357 (33; ± 1.565; ± 0.35)	0	1-6		0.013 (0.162; ± 0.013; ± 0.018)	0	0.0001-0.0448
Other	4.29 (-0.57, 2.57)	1 (3; ± 0.535; ± 0.202)	0	1		0.071 (0.212; ± 0.018; ± 0.002)	0	0.017-0.118

TO BE SUBMITTED AS A SHORT NOTE

CHAPTER 3: ENTANGLEMENTS OF BIRDS IN FISHING GEAR IN SOUTHERN PORTUGAL FROM 2010 TO 2019

Silvia Rao[1], Katy R. Nicastro[1,2], María Casero[3], Christopher D. McQuaid[2], Gerardo I. Zardi[2]*

[1]CCMAR-CIMAR – Associated Laboratory, University of Algarve, Campus de Gambelas, Faro 8005–139, Portugal

[2]Department of Zoology and Entomology, Rhodes University, Grahamstown 6140, South Africa

[3]Wildlife Rehabilitation and Investigation Centre (RIAS) – Associação ALDEIA, Ria Formosa Natural Park, Olhão, Portugal

*Corresponding Author: Email: zardi73@yahoo.it

3.1 Abstract

Entanglement in fishing gear is an increasing threat to wildlife. Temporal trends of fishing gear entanglements in 14 aquatic and two marine bird species were assessed in this studyin the south of Portugal, over a period of ten years (2010-2019). The records were taken from the database of a wildlife recovery center (RIAS) in southern Portugal. A total of 256 individuals out of 5843 were recorded to be affected by entanglements. The most affected species were the two aquatic species *Sterna caspia* (100%) and *Larus marinus* with 50%. Entanglements mainly resulted from fishing lines with and without hooks and fishing netsSignificant temporal trends were observed for *Phalacrocorax carbo* and *Morus bassanus,* decreasing and increasing, respectively. This study sets a first multispecies baseline for incidence of fishing gear entanglements of aquatic birds in southern Portugal. Entanglement baseline data and assessment of temporal trends are important to estimate changes through time and among regions, and to adapt management actions.

3.2 Main text

Contamination of the marine environment with anthropogenic litter, especially that made of plastic, is prevalent worldwide and an ever-increasing environmental threat affecting marine wildlife from the most populated and heavily urbanised beaches to the most remote and pristine islands (Willoughby et al. 1997; Uneputty and Evans 1997; Williams et al. 2017; Ríos et al. 2018; Velez et al. 2019).

As anthropogenic waste spreads and accumulates throughout the environment, it poses a major threat to a rising number of marine taxa- including cetaceans, turtles, fish, crustaceans and seabirds- influencing their behaviour, physiology and survival (Jagiello et al. 2019).

Animals are exposed to litter mainly through ingestion and entanglement (Laist 1987; Gall and Thompson 2015; Hammer et al. 2016; O'Hanlon et al. 2017; Provencher, Bond, and Mallory 2015; Provencher et al. 2017).

Ingestion can be unintentional, while individuals feed on other prey items, or deliberately, when materials are erroneously identified as food (Laist 1987; Cadée 2002). Entanglement is generally passive, when individuals get trapped in dispersed debris materials such as fishing or plastic bags, or it may also be active, when individuals get stuck in materials that they gather intentionally (Gregory 2009; Phillips et al. 2010).

Seabirds are especially exposed to the increasing presence of anthropogenic materials in the environment (Battisti et al. 2019b).

The most recent review suggests that at least 40% of all seabird species contain ingested plastic, and 25% have been recorded entangled in plastic (Gall and Thompson, 2015; Kühn et al., 2015; Ryan, 2016). Entanglement of birds is more noticeable than ingestion, as are its effects, such as wounds, hindered mobility (with significant implications for the capacity to feed or avoid predators) and drowning (Laist, 1997; Kühn et al., 2015).

Evidence strongly indicates that almost all species are exposed to entanglement risk, and that with increasing survey effort, the number of affected species is destined to rise. However, many entangled birds remain undetected because they often die far from the shores (Laist, 1997). It is thus likely that the number of entangled birds is largely underestimated (Laist, 1997; Kühn et al., 2015). In fact, detailed reviews of entanglement records for marine organisms, confirm that the proportion of impacted seabirds is appreciably higher than previously estimated (Kühn et al. 2015), up from 25% to 36% (Ryan 2018).

Recent entanglement records obtained from > 30 countries, show that fishing lines and netting are responsible for most entanglements. Until the 1960s, natural fibres such as cotton and hemp were the main materials used to make fishing gears. Later, these have been replaced by synthetic materials which eventually results in the accumulation of long-lasting fishing gear waste (Gregory 2009). As a consequence, entanglement in abandoned, lost or discarded fishing gear has become an increasing menace to wildlife. Currently, it is estimated that 88% of seabird species entangled in fishing gear. Fishing line entangled a greater proportion of species than netting, 78% and 36% respectively (Ryan 2018).

Critically, the proportion of entangled species does not share light on the demographic impact of entanglement, which should be assessed as the proportion of entangled individuals within species. Such a lack of information and, most of all scarcity of records, make a realistic assessment of temporal and spatial changes in the rate of entanglement difficult. The limitations are particularly relevant in regions where baseline studies are not yet available. Baseline data not only are central to assessing changes through time and differences among regions, but they are also important for an effective definition of management and conservation efforts.

Relative to northern Europe, in southern European countries, attempts to assess the impact of anthropogenic litter in aquatic birds have so far been limited (i.e., Codina-García et al., 2013). Only recently it has been shown *Larus michahellis, Larus fuscus, Ciconia ciconia* and *Morus bassanus* are, even if in different proportions, affected by plastic ingestion in Portugal (Nicastro, Savio, et al. 2018; Basto et al. 2019), it was also observed that the nests of *Larus michahellis* found in urban areas are highly impacted by anthropogenic materials (Lopes et al. 2020).

Currently, there is no published information concerning entanglement in aquatic birds in Portugal.

This paper attempts to give an overview of bird species recorded to be affected by fishing gear entanglement in the southern regions of Portugal (Algarve and Alentejo) from 2010 until 2019. The southern coast of Portugal is surrounded by the, in 1987 classified, Natural Park of the Ria Formosa, which has a large Aves biodiversity and reaches around 100 different bird species every year (Amaral 2009a; Farinha and Costa 1999). The lagoon was identified to provide relevant ecological functions and ecosystem services, especially feeding, breeding and nursery areas, from which local fisheries might benefit (Ribeiro et al. 2006; Guimarães et al. 2012). This environment has been acknowledged to be of great economic, cultural and social value.

The samples in this study were provided by a wildlife recovery center (Centro de Recuperação e Investigação de Animais Selvagens - RIAS). During this examination, the species of interest were: *Ciconia ciconia, Larus fuscus, Larus michahellis, Morus bassanus, Phalacrocorax carbo, Ardea cinerea, Arenaria interpres, Calidris alpina, Egretta egretta, Gallinula chloropus, Larus audouinii, Larus marinus, Pandion haliaetus, Phoenicopterus ruber, Platalea leucorodia, Pluvialis squatarola* and *Sterna caspia.*

In records involving bird entanglements, it is challenging to differentiate between individuals caught by active or ghost fishing gear, as it is difficult to understand if birds entangled in fishing lines were previously caught in abandoned lines or in active gear (Ryan 2018, Taylor 2004; Abraham et al. 2010). In this study samples that had ingested hooks were not treated as entanglements. As entanglements, individuals affected by fishing lines with ingested hooks, fishing lines and fishing nets were identified.

For each species, the percentage of individuals affected by entanglements was calculated over the ten years. Further, it was observed, how many of those were released or died due to injuries. Because many species did not have enough samples over the years, only for five species a trend over time was graphically plotted. In total, over the ten years, n = 256 individuals arrived at the center because of entanglements in fishing gear. Fishing lines and nets are usually found coiled around the limbs. In the most common cases the injuries commence in the distal region of the entanglement area with cold oedema due to the lack of blood supply, followed by tissue necrosis resulting in the death of the affected limb. In the entanglement pressure area, the injuries resulted in lacerations and cuts of the tissues. Indirectly, due to the impossibility of developing normal feeding habits, poor body condition may be seen in animals entangled in lines or nets for a long period. The prognosis of the animal mostly depends on how long the entanglement has taken place until medical care is provided. Poor body condition was the main cause of natural death of the animals after admittance. Severe injuries in the thoracic limbs of birds that prevent flying and, consequently, the release of the animal, will lead to euthanasia. In the pelvic limbs, when complete recovery of the member is not possible, most of the species can live with different levels of limb amputation or even without one limb. Less frequently, two limbs are affected, and the animal is not able to recover mobility, then, euthanasia is needed. The chance to be released will depend on the affected limb (thoracic or pelvic), the biology and ecology of the species and its ability to live with the aftermath.

Larus michahellis had the highest number of individuals reaching the center (124 individuals) due to entanglements, but only 3.9% of the total animals reaching the center over the ten years were affected by entanglements (Figure 3.1 B). The frequency of occurrence (FO%) showed a slight decrease over time (Spearman´s Rho: - 0.359; Figure 3.1 A). Of the 124, 67.7% died due to the injuries resulting from the entanglements.

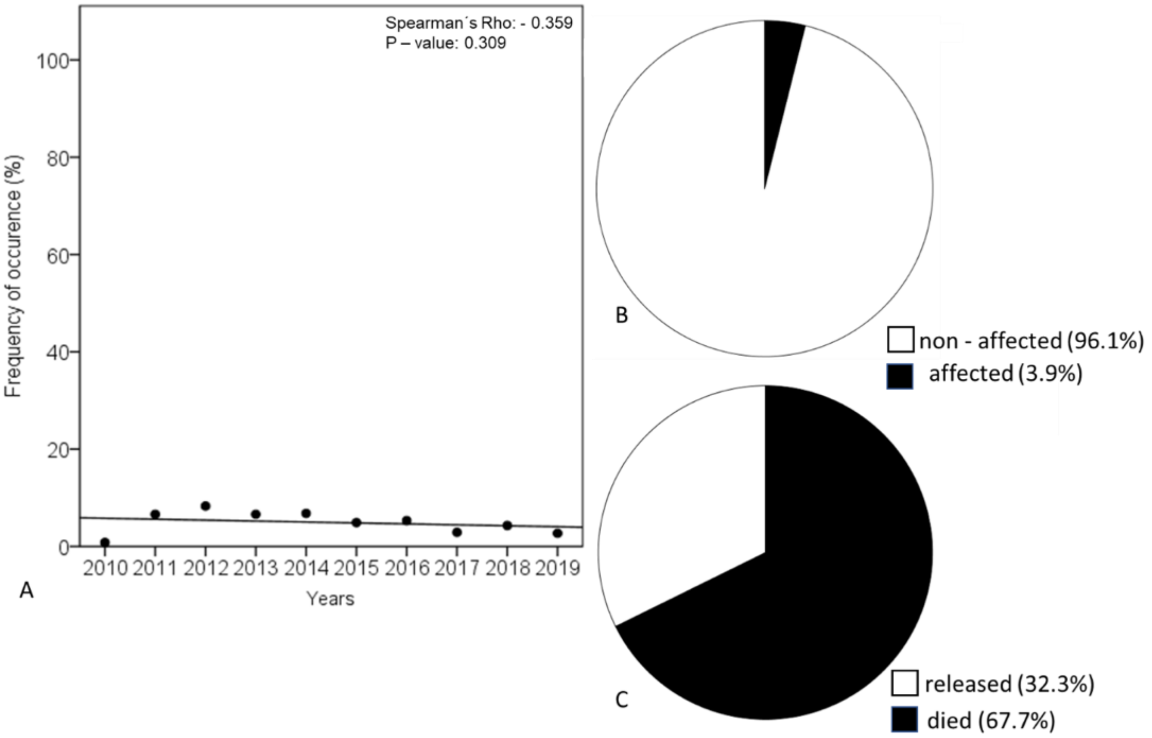

Figure 3.1: *Larus michahellis* (n = 124); (A) Frequency of occurrence (%), (B) Percentage of affected individuals compared to the total numbers reaching the recovery center; (C) Fate of affected individuals.

Also, *Larus fuscus* had a slight decrease in the FO% over time (Spearman´s Rho: - 0.467; Figure 3.2 A). 1.2% of all *L. fuscus* reaching the center from to 2010 from 2019 resulted to be affected by entanglement (Figure 3.2 B). Of the specimens processed, 68.4% died due to the injuries resulting from the entanglements.

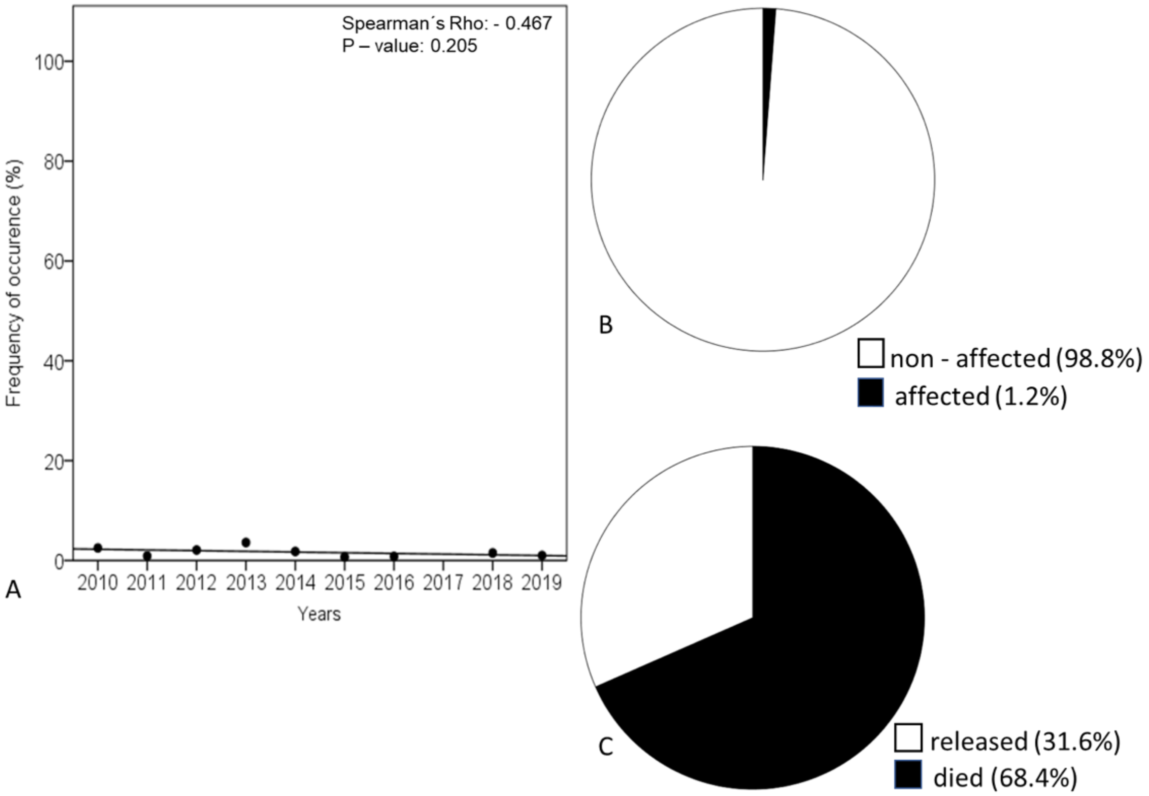

Figure 3.2: *Larus fuscus* (n = 19); (A) Frequency of occurrence (%), (B) Percentage of affected individuals compared to the total numbers reaching the recovery center; (C) Fate of affected individuals.

Phalacrocorax carbo had a significant decrease over time in the FO% (Spearman´s Rho - 0.886, P-value:0.01; Figure 3.2 A). 36.1% of all *P. carbo* reaching the recovery center were entangled in fishing gear and 41.7% died or were euthanised due to the injuries.

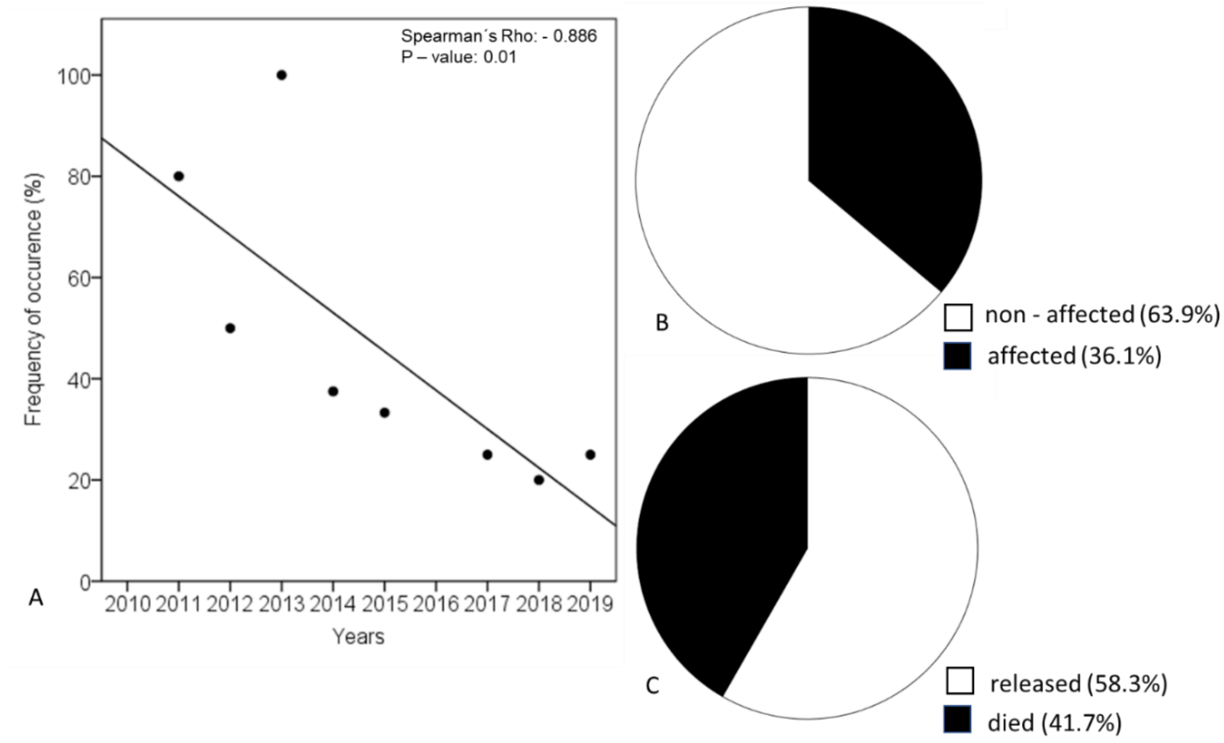

Figure 3.3: *Phalacrocorax carbo* (n = 12); (A) Frequency of occurrence (%), (B) Percentage of affected individuals compared to the total numbers reaching the recovery center; (C) Fate of affected individuals.

Morus bassanus had a 0.01 level of significance in the Frequency of occurrence (F0%; Figure 3.4 A) over the years 2015 and 2019, with a correlation coefficient of 1.0. 18.3% of all *M. bassanus* treated at the center were due to entanglements and 89.7% died due to the injuries (Figure 3.4 B and C).

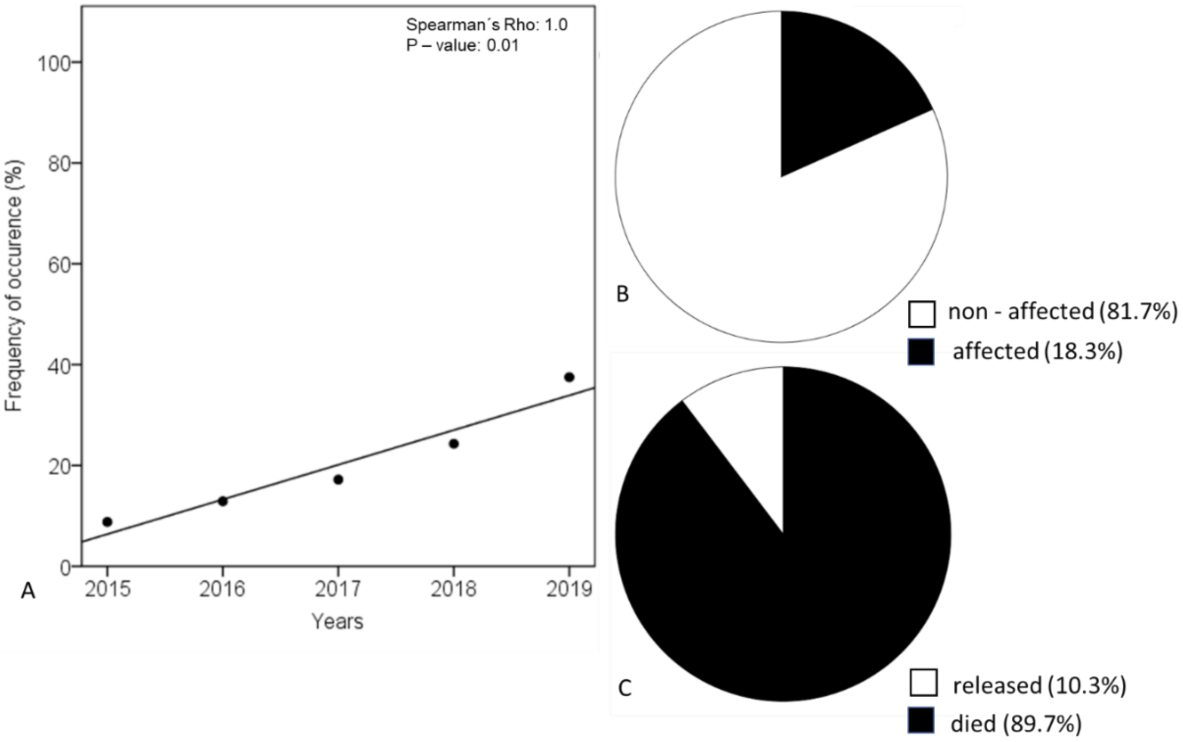

Figure 3.4: *Morus bassanus* (n = 58); (A) Frequency of occurrence (%), (B) Percentage of affected individuals compared to the total numbers reaching the recovery center; (C) Fate of affected individuals.

Ciconia ciconia had a significance level of 0.05 in the frequency of occurrence (FO%), with a Pearson correlation of 0.745 (Figure 3.5 A). 4% of all *C. ciconia* entering the center over the ten years were affected by fishing gear entanglements, from that 75% died (Figure 3.5 B and C).

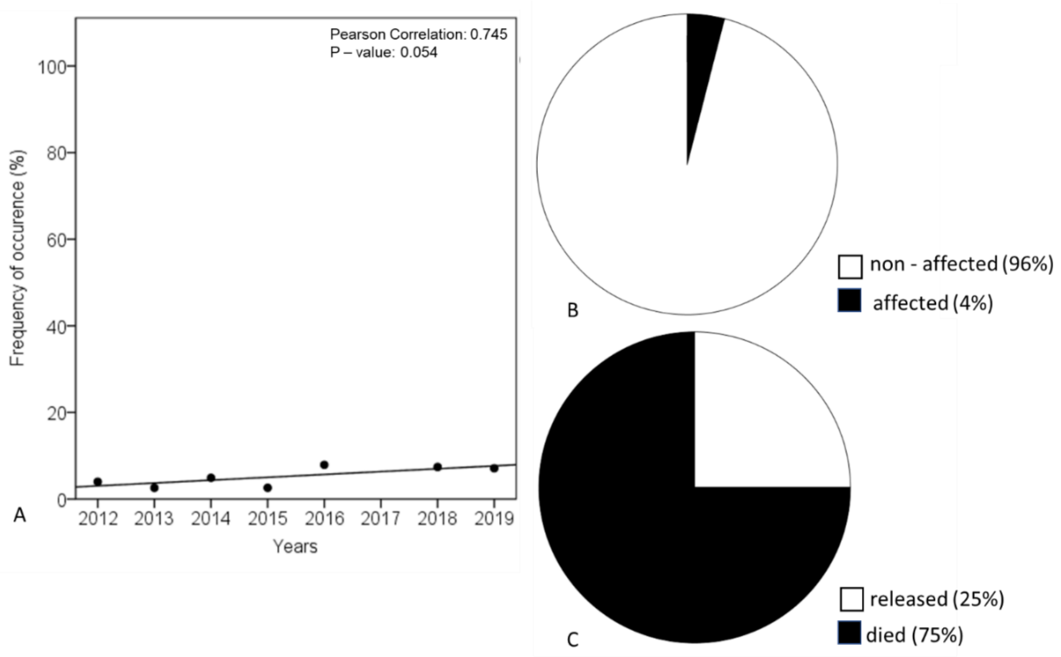

Figure 3.5: *Ciconia ciconia* (n = 16); (A) Frequency of occurrence (%), (B) Percentage of affected individuals compared to the total numbers reaching the recovery center; (C) Fate of affected individuals.

The other eleven species did not have enough samples over the years to conduct an in-depth examination. Nevertheless, the rate per species affected by entanglements compared to the total number reaching the recovery center was calculated. Further, it was observed how many of those were released or died due to the injuries. Overall, it can be stated that most individuals died because of the injuries caused by the entanglements. For *Calidris alpine, Gallinula chloropus*, *Pluvialis squatarola*, *Platalea leucorodia*, *Phoenicopterus ruber* and *Sterna caspia* mortality rate was of 100% (Figure 3.6). Proportionally the most affected species by entanglements out of the species with temporal trends were *Sterna caspia* (100%; Figure 3.6 L) and *Larus marinus* (Figure 3.6 H) with 50%, whereas the least affected were *Larus audouinii* (Figure 3.6 F) with 2.4% followed by *Gallinula chloropus* (4.2%; Figure 3.6 E) and *Phoenicopterus ruber* (5.9%; Figure 3.6 J).

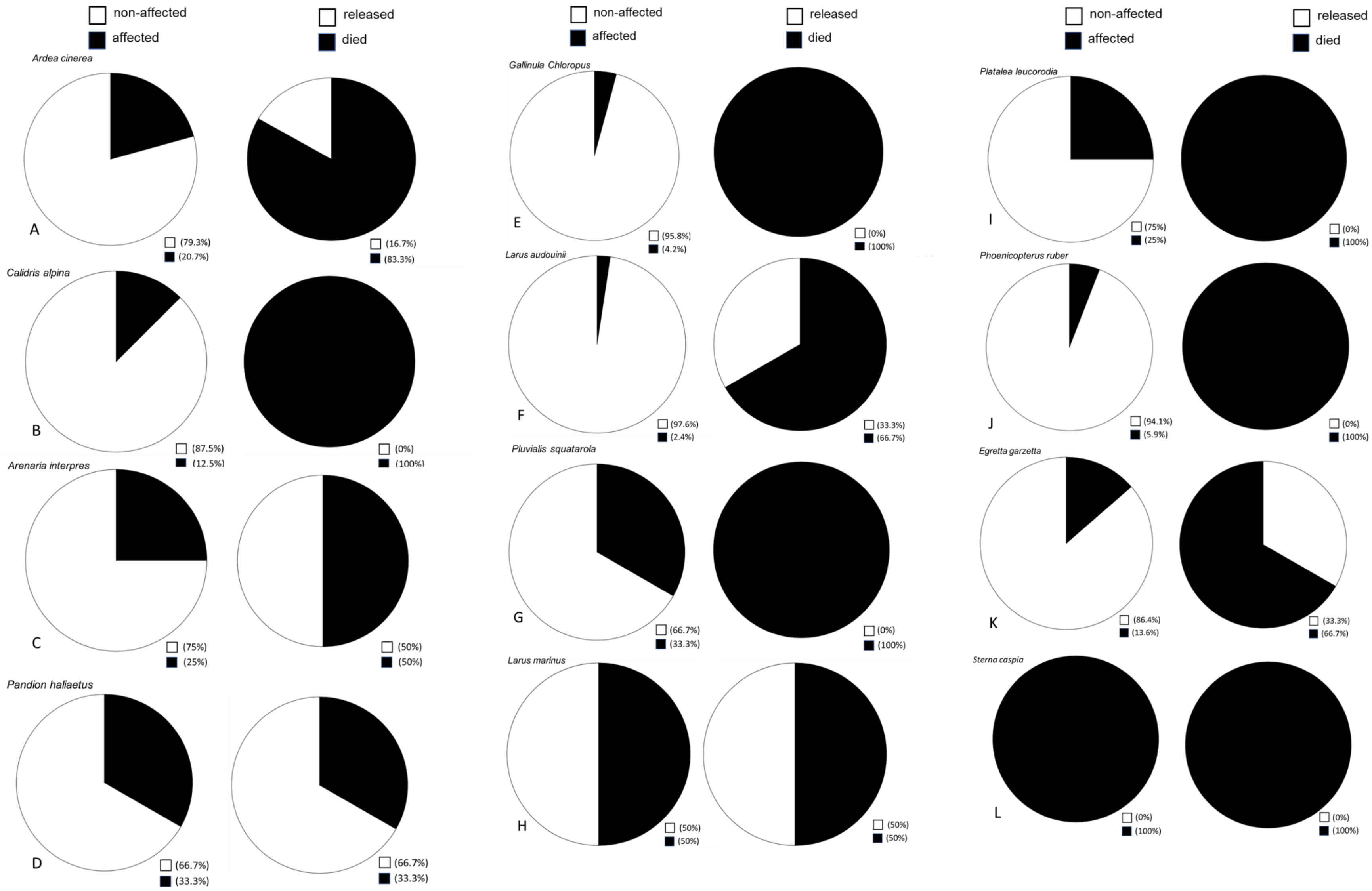

Figure 3.6: Left: Percentage of affected individuals compared to the total numbers reaching the recovery center (non-affected/affected); Right: Fate (released/died) of affected individuals**.** *Ardea cinerea* (A), *Calidris alpine* (B), *Arenaria interpres* (C), *Pandion haliaetus* (D), *Gallinula chloropus (E)*, *Larus audouinii* (F), *Pluvialis squatarola (G)*,*Larus marinus* (H), *Platalea leucorodia* (I), *Phoenicopterus ruber* (J), *Egretta egretta* (K) and *Sterna caspia* (L).

Interestingly, the results show that different species did not show consistent patterns in terms of the percentage of individuals affected by entanglement and of those that suffered lethal consequences. This outcome highlights the crucial importance of multispecies assessments. As for plastic ingestion, examinations for an extensive range of species (including non-indicator species) are central to capturing the pervasiveness of plastic entanglement and recognising factors that account for differences in the quantities and qualities of plastic affecting different species (McIntosh et al. 2015; Stelfox et al. 2016). Moreover, comprehensive multi-species surveys may also prove valuable in identifying alternative species monitoring actions (e.g. Acampora et al. 2016). Understanding the drivers of interspecific differences is difficult. Evidence from numerous studies from different regions and species indicates that behavioural factors (e.g. migratory and feeding strategies), amount of plastic pollution in the foraging or nesting environments are at play in determining the frequency of plastic entanglement (Belant et al. 1998; Jagiello et al. 2018; Bond et al. 2012; Thompson et al. 2020; Lopes et al. 2020). Understanding such complex dynamics will require *ad hoc* studies testing the intricate effects of multiple biotic and abiotic determinants and their variation in time and space.

Species for which more data are available, such as gulls, storks and gannets in our case, allow for interpretation and speculations of the results that extend beyond species or location specificity. Here, it is shown that while no significant changes through time were observed for *L. michahellis, L. fuscus* and *C. ciconia,* significant trends were observed for *P. carbo* and *M. bassanus,* decreasing and increasing respectively. Another conspicuous difference is that *P. carbo,* while experiencing much higher frequencies of entanglement, suffered relatively lower resulting deaths compared to the other species for which temporal fluctuations were described. All these species have been reported incorporating marine debris, often lost fishing gear, into their nests at increasing rates (Montevecchi 1991; Votier et al. 2011; Bond et al. 2012; Jagiello et al. 2019; Tavares et aal. 2019; O'Hanlon et al. 2017; Battisti 2020; Lopes et al. 2020; Thompson et al. 2020) and it has been shown that adults can suffer severe injuries such as broken wings and legs as a consequence of nest entanglement (Kwieciński et al. 2015; Votier et al. 2011; Seacor et al. 2014). There is evidence that the nature of anthropogenic debris found within nests can vary significantly interspecifically and within the same species in different regions (Votier et al. 2011; Tavares et al. 2019). These important observations suggest that despite litter incorporation in nest seems to be widespread, different litter size and shape may be responsible for the observed distinct frequencies of entanglement. Another potential cause of distinct entanglement frequencies and mortalities can be found in different foraging strategies. Indeed, feeding behaviours and ranges vary greatly in the species assessed in this study. For example, while cormorant and gannets are both diving species; cormorants make use of specialised hunting techniques, characterised by brief short-distance chase and rapid neck extension and visual prey detection at short range (Grémillet et al. 1998; Martin et al. 2008). In contrast, a relatively lower portion of gannets engage in underwater prey pursuit while mostly relying on capturing the prey as an immediate consequence of direct plunging. Feeding range may also be a key determinant exposing the two species to different entanglements rate. In fact, while cormorants mainly feed within coastal and estuarine habitats, gannets are known to exploit more offshore areas (Hamer et al. 2001). Taken together, such key behavioural differences (hunting techniques and ranges) may explain the contrasting trends and patterns observed in the two species. Indeed, entanglement of gannets in heavy ropes or nylon fibres from beam trawler nets has been reported numerous times. Further, several gannets were found dead with broken or missing wings or broken mandibles most likely as a result of being pulled out of a net on a fishing trawler (Bartle 1991; Weimerskirch et al. 2000; Sullivan et al. 2006; Watkins et al. 2008; Abraham 2010).

Unexpectedly, despite the recent evidence supporting the increasing feeding on anthropogenic litter as well as the increasing incorporation of litter in their nests, the examined gull species did not display an increasing trend over time (Nicastro, Savio, et al. 2018; Basto et al. 2019; Lopes et al. 2020). In *L. michahellis* the incorporation of anthropogenic litter in the nests largely depended on the amount of litter in the colonised areas with significant differences between urban areas and less populated ones (Lopes et al. 2020). Species close to coastal areas are more susceptible to be affected by fishing material, in fact, coastal colonies of kelp gull species were found to integrate fishing lines and fishing ropes in their nests (Witteveen et al. 2017). Because of the year-round fishing activities and the vicinity to urban areas in the area of investigation, we predicted an increment in the trend of the gull species over time and we also assumed a significant increment over time in *C. ciconia*, because of the dominant influence anthropogenic litter has on their feeding and nesting habits (Djerdali et al. 2016; Nicastro et al. 2018; Jagiello et al. 2018). In fact, a slight increment over time was observed, although not significant.

Because entanglement is characterised by a narrower suite of plastic items compared to plastic ingestion, more focused mitigation actions can be implemented. Such actions may include banning of high-risk activities and educating users to properly discarding high-risk materials such as fishing gear. There are examples showing that this can be achieved by providing, for instance, specific fishing gear bins in combination with educational signage campaigns (Ryan 2018).

As the presence of fishing gears continues to increase in coastal and aquatic environments, our data will provide a crucial record of entangled species and a basis from which to survey long-term trends in plastic entanglement, particularly for Portuguese monitoring programs for which data remain scarce.

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