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EXPOSURE TO MICROPLASTICS IN PELAGIC AND COASTAL SEABIRDS FROM TEMPERATE AND TROPICAL ENVIRONMENTS

Dissertation in Msc in Ecology, supervised by Prof. Dr. Jaime Albino Ramos and Dr. Vitor Hugo Paiva and presented to the department of Life Sciences, Faculty of Sciences and Technology of the University of Coimbra.

September 2022

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1 2 9 0 FACULDADE DE CIÊNCIAS E TECNOLOGIA UNIVERSIDADE D COIMBRA

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I declare that this thesis was written and organized by me, and I confirm that it has not been previously submitted, in whole or in part, to obtain another academic degree. I confirm that the work described was mostly done by me, and other contributions are clearly acknowledged in the text with appropriate references. I performed all laboratory and field work except Cabo Verde sampling collection done by Diana Matos. All data analysis and thesis writing was done by me

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Abstract

Plastic is currently the most common anthropogenic material in the marine environment. The study of the presence and quantity of plastics in the ocean is an increasingly discussed topic. However, studies comparing Portugal with Cabo Verde are lacking. In this study faecal samples were analysed with the main objective of evaluating the role of taxonomy, feeding ecology and distribution on the plastic ingestion of Portugal and Cabo Verde seabird species.

Seven seabird's species were studied in this project: Audouin´s gull (*Ichthyaetus audouinii*), Cory's shearwater (*Calonectris borealis*), Cape Verde Shearwater (*Calonectris edwardsii*), Red-billed Tropicbird (*Phaethon aethereus*), Brown booby (*Sula leucogaster*), Bulwer´s Petrel (*Bulweria bulwerii)* and Boyd´s Shearwater (*Puffinus lherminieri boydi*). The samples were collected in Portugal (Audouin´s gull and Cory's shearwater) and in Cabo Verde (Cape Verde Shearwater, Red-billed Tropicbird, Brown booby, Bulwer´s Petrel e Boyd´s Shearwater) between February and September 2021.

The presence of plastics was analyzed using faeces as a proxy for ingestion. All species showed presence of plastics (*Ichthyaehus audouinii* = 62.5% of Frequency of Ocurrence; *Calonectris borealis* = 41.9%; Calonectris edwardii = 86.5%; *Phaethon aethereus* = 64.7%; *Sula leucogaster* = 68.5%; *Bulweria bulwerii* = 35.9%; *Puffinus iherminieri boydi* = 44.4%).

This study showed that the presence of plastics in the studied species was not only influenced by the seabird's taxonomy, but is also driven by intrinsic and extrinsic factors such as bird size, habitat, foraging area and interaction of seabirds with fishing activities.

Results from this study provide more evidence to our growing perception on the ubiquity of plastic pollution in the marine environment and further supported the usefulness of using seabirds as sentinels of plastic pollution in both neritic and oceanic regions.

Keywords:
Cabo Verde; Faecal Samples; Ingestion; Plastic Pollution; Portugal.

Resumo

Plástico é, atualmente, o material antropogénico mais comum no ambiente marinho. O estudo da presença e quantidade de plásticos no oceano é um tema cada vez mais abordado, porém o seu estudo comparando Portugal com Cabo Verde não é muito aprofundado. Neste estudo foram analisadas amostras de fezes com o objetivo principal de avaliar o papel da taxonomia, ecologia alimentar e distribuição na ingestão de plásticos por parte de espécies de aves marinhas de Portugal e Cabo Verde.

Sete espécies de aves marinhas foram alvo de estudo nesta tese, sendo estas a Gaivota de Audouin (*Ichthyaetus audouinii*), Cagarra (*Calonectris borealis*), Cagarra de Cabo Verde (*Calonectris edwardsii*), Rabo de palha de bico vermelho (*Phaethon aethereus*), Ganso patola (*Sula leucogaster*), Bulweria (*Bulweria bulwerii)* e Pardela de Cabo Verde (*Puffinus lherminieri boydi*). As amostras destas espécies foram recolhidas em Portugal (Gaivota de Audouin e Cagarra) e em Cabo Verde (Cagarra de Cabo Verde, Rabo de palha de bico vermelho, Ganso patola, Bulweria e Pardela de Cabo Verde) entre fevereiro e setembro de 2021.

A presença de plásticos foi analisada usando fezes como *proxy* de ingestão. Todas as espécies apresentaram presença de plásticos (*Ichthyaehus audouinii* = 62.5% de frequência de ocorrência; *Calonectris borealis* = 41.9%; Calonectris edwardii = 86.5%; *Phaethon aethereus* = 64.7%; *Sula leucogaster* = 68.5%; *Bulweria bulwerii* = 35.9%; *Puffinus iherminieri boydi* = 44.4%).

Os resultados deste estudo mostraram que a presença de plásticos nas espécies em estudo não depende apenas da taxonomia das aves marinhas, mas também de fatores intrínsecos e extrínsecos tais como o tamanho da ave, habitat, ecologia alimentar e interação das aves com atividades piscatórias.

Os resultados deste estudo forneceram mais evidências para a crescente perceção da omnipresença de plástico no ambiente marinho e apoiou, ainda mais, a utilidade do uso de aves marinhas como sentinelas da poluição por plásticos em regiões neríticas e oceânicas.

Palavras-chave:

Cabo Verde; Amostras Fecais; Ingestão; Poluição por Plásticos; Portugal.

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List of Abbreviations

United Nations Environment Program (UNEP)
Municipal solid waste (MSW)
Persistent organic pollutants (POPs)
Audouin´s gull (IA)
Cory’s shearwater (CB)
Cape Verde Shearwater (CE)
Red-billed Tropicbird (PA)
Brown booby (SL)
Bulwer´s Petrel (BB)
Boyd´s Shearwater (PB)
Internacional Unit for Conservation of Nature (IUCN)
United Stated of America (USA)
generalized linear model (GLM)
Akaike’s Information Criterion (AIC)
Non-metric multidimensional scaling (NMDS)
Nanoplastics (NP)
Small microplastics (SMP)
Large microplastics (LMP)
Mesoplastics (MP)
Frequency of occurrence (F.O.)
Fourier-transform infrared spectroscopy (FTIR)

CHAPTER I - Introduction

1.1 Plastic pollution: the new threat

A simple definition of plastic is a cheap, lightweight synthetic or semi-synthetic organic polymer (chain of molecules linked by carbon, hydrogen, oxygen, and/or silicon) that is highly resistant to erosion (Halden, 2010). To this definition should be added the fact that plastics are now the most common type of anthropogenic material found in surveys globally. No wonder that plastic pollution has become one of the most important environmental concerns nowadays and is a “hot topic” in environmental pollution research. The United Nations Environment Program (UNEP) defined this as a critical problem and refer to it as an important threat as climate change.

The global production of plastics in the 1950s was about 1.5 million tons, but currently it is estimated to be 300 million metric tons annually (Andrady, 2017). Since plastic is extremely useful the prediction is that plastic production will continue to increase.

There is a direct relation between the quantity of plastics produced (micro and macro) and the increase in human population (Fig. 1). Depending on the location, 10 - 15% of Municipal Solid Waste (MSW) is plastic, with some of this fraction ending in the oceans as pollution (Andrady, 2017). The majority of plastic produced is for domestic use or fishing purposes, some examples are packaging, polyester fibers from clothes and fishing nets. The worst scenario is that a large percentage of plastics produced are for single use, such as straws and packaging, which means that after the first use it becomes useless litter.

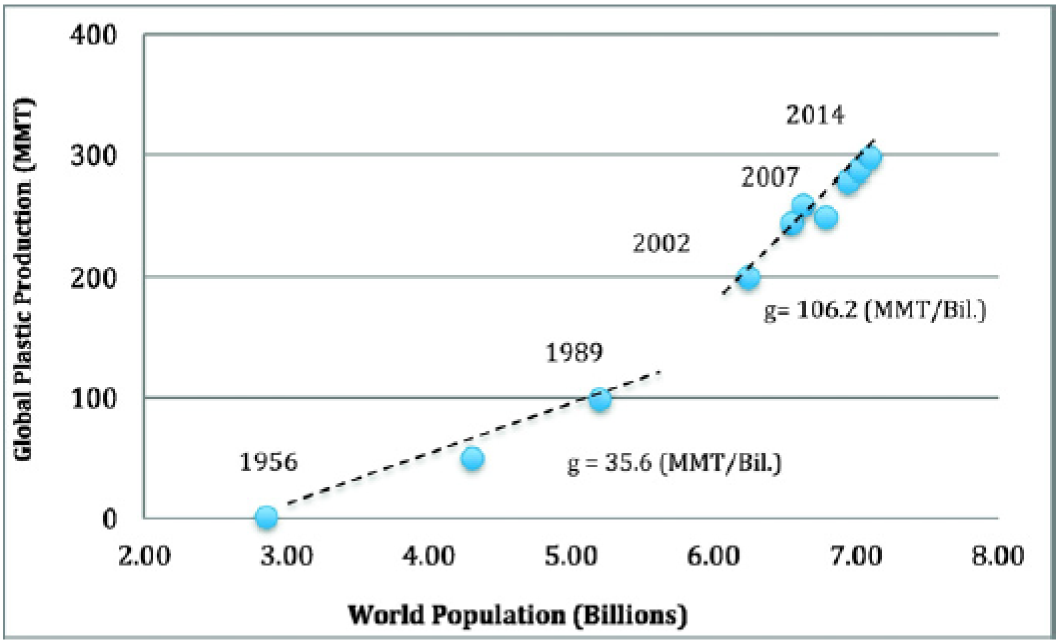

Figure 1. The change in global production of plastics with the population. MMT: million metric ton (Andrady, 2017).

In terms of size, plastics can be divided into macroplastics (>2.5 cm), mesoplastics (5 mm - 2.5 cm), microplastics (1 µm - 5 mm) and nanoplastics (<1 µm) (GESAMP 2015) as shown in table 1.

Table 1. Classification of plastic debris in the environment (GESAMP 2015).

CLASS	SIZE RANGES	VISUALIZATION
MACROPLASTICS	2.5 cm – 1 m	Naked eye
MESOPLASTICS	5 mm – 2.5 cm	Naked eye or optical microscope
MICROPLASTICS	1 µm – 5mm	Optical microscope
NANOPLASTICS	1nm – 1 µm	Electron microscope

Macroplastics have a higher recognition by media and the society in general because they are the larger plastics, and people can find them in their daily lives, commonly in beaches. They are frequently ingested by animals such as seabirds, fishes, cetaceans and even mammals.

Microplastic is a term that is used for a wide range of dimensions, varying from 1 µm to 5 mm. There are two types of microplastics: the ones produced originally micro sized (primary microplastics) and those that were macro sized but fragmentated into micro sized plastics (secondary microplastics). Primary microplastic are essentially produced for cosmetic and medical purposes.

Secondary microplastic are derived from macroplastics by degradation. This process can occur on land and on the ocean. The degradation occurs by mechanical, biological and chemical factors (Westphal *et al*. 2008).

An important concern towards microplastics is that they are more likely to be ingested by marine organisms and given the fact that they can be contaminated with persistent organic pollutants (POPs), be transferred to the upper organisms on the food chain and therefore more harmful for marine food webs.

1.2 Plastic pollution in the marine environment: from coastal areas to the deep sea

Studies examining marine and freshwater species interaction with plastics have increased rapidly over the last several decades (Provencher *et al*. 2019), because this phenomenon has become more concerning and more ubiquitous. The majority of plastic accumulated in the ocean derive from human activities or are due to natural causes. Human activities such as tourism, activities within large metropolitan areas and errors at transportation and manipulation of microplastics are important in terms of abundance and distribution of plastic debris (Galgani *et al*. 2000). Natural phenomena such as storms and floods may also apport high concentration of plastic to the ocean (Barnes *et al*. 2009).

Eighty percent of microplastics accumulated in the ocean came from land and are transported into the ocean (Derraik, 2002). The high concentration of microplastics in the ocean normally peaks in the surroundings of industrial areas (Stamper *et al*. 2012). The microplastics from oceanic origin derive from fishing activities and fishing shipwrecks (Li *et al*. 2016). Debris accumulation and distribution in the marine environment is influenced by many factors. These can include the wind, currents, geography and bathymetry of the area, and some other anthropogenic factors like points of entry, distance from population centers and oceanic trade routes (Barnes *et al*. 2009). Due to its resistance to degradation, most plastic debris will persist in the environment for centuries, which makes plastics the most omnipresent pollutant, not just because it is produced worldwide but also because it is transported very easily by wind or ocean currents or even transported by humans. Plastics can only float in the ocean due to their low density (Andrady, 2011). Recent surveys found microplastics at the bottom of the ocean (Cole *et al*. 2011). This happens because a microbial biofilm may involve the plastic particles, allowing a colonization by algae and invertebrates and increasing the plastic density which will make them sink into the ocean.

Although plastic is globally consumed, the waste mismanagement and pollution varies among countries (Fig. 2). An estimated value between 4.8 to 12.7 million tons of plastic waste was released into marine waters in 2010. The disparity between countries is due to each country different values of plastic usage, coastal population density and the waste management practices. A marked example is the comparison between the United Stated of America (USA) and India, which have similar coastal population density. However, even though the American country has a much higher waste quantity produced per day, 88% of the Indian waste suffers no treatment versus just 2% of waste mismanagement in the USA, which results in a higher contribution of marine plastic production by India than by the USA (Worm *et al*. 2017).

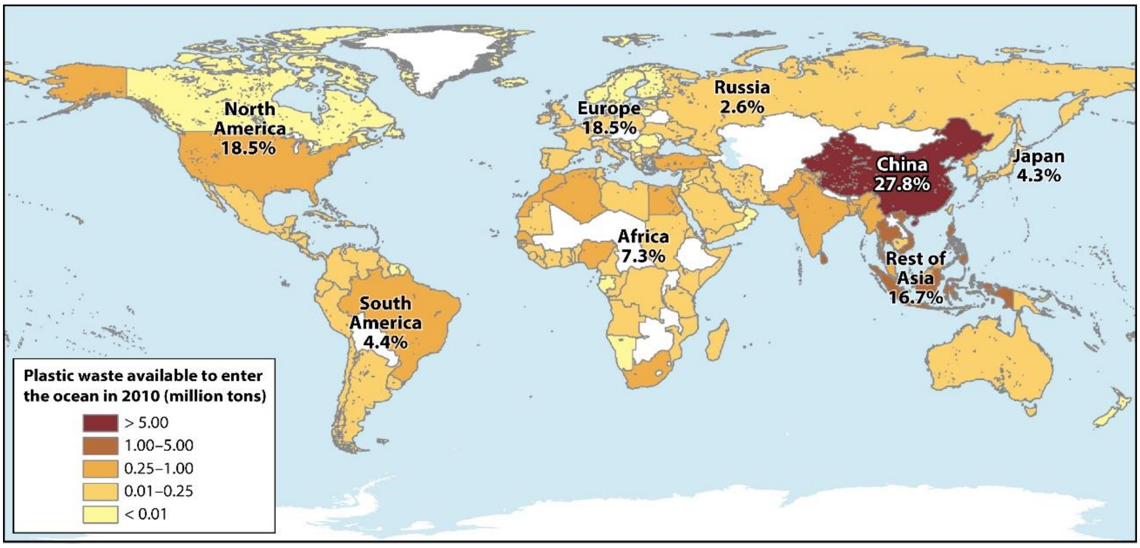

Figure 2. Worldwide patterns of plastic production and pollution. The percentage is each region's contribution to the global plastic production. The colour shows the estimated mass of mismanaged plastic waste in million tons in 2010 (Worm *et al*. 2017).

Limiting research on the impacts of plastic pollution on the ocean surface may provide only a very partial view about the severity of this problem.

1.3 How marine megafauna can be affected by plastic pollution

While the weight fraction of microplastics in total plastic litter is relatively low, they are able to interact with a wide variety of marine organisms, ranging from the smallest (zooplanktons) (Ferreira *et al*. 2016) to the largest (fin whales (*Balaenoptera physalus*)) (Fossi *et al*. 2016). Most of the knowledge about plastic threats has been acquired from studies on the marine environment, mainly nearshore and coastal regions. In these areas several studies noticed wildlife entangled or ingesting plastic (Li *et al*. 2016; Provencher *et al*. 2017). The literature search for this thesis focused chiefly on seabirds, marine mammals and sea turtles (*Cheloniidae*); all these organisms are very mobile providing information of a very wide area, but the consequence is that it is very difficult to know exactly the location where the organism had contact with the debris.

The known plastic-marine biota interactions are ingestion, entanglement and chemical effects.

Ingestion

Macro and microplastics are both ingested by wildlife, but the ingestion rarely induces immediate death, it is more common to provoke chronical problems that may lead to slow death because marine biota may be affected by different pathways as intestinal tract blockage or puncture, gastric enzymes secretion inhibition, less steroid hormones production, tissue inflammation, growth reduction or even reproductive incapacity and lack of appetite (Azzarello *et al*. 1987; McCauley *et al*. 1999; Wright *et al.* 2013). However, some animals such as gulls (*Laridae*) can regurgitate plastics decreasing the negative effects of the previous ingestion (Codina-garcía *et al*. 2013).

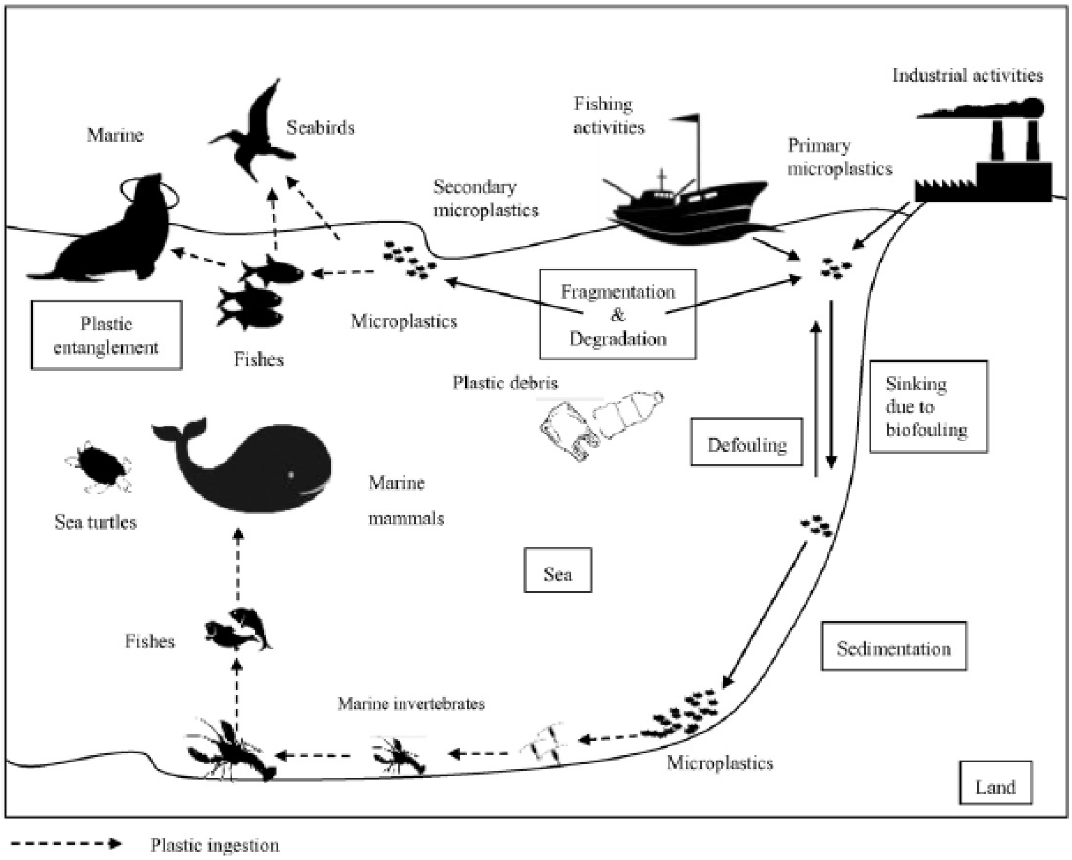

Figure 3. Potential pathways of plastic debris and its biological interactions. All the organisms presented already been found contaminated with plastics (Wright *et al.* 2013; Ivar Do Sul *et al*. 2014).

Plastic entanglement

This has been reported since 1980s (Laist, 1987), and some studies argue that more organisms are affected by entanglement than by ingestion (Gall *et al*. 2015). After getting entangled at the organism, the plastic can aggregate even more materials leading to the increase of the energy needed to do all the activities and consequently increases the need of food. The materials that most often cause entanglement are packaging and fishing materials (nets and ropes) (Li *et al*. 2016). Entanglement is mostly influenced by the behaviour of the organism. As a consequence of entanglement by plastic the animal can die by drown, suffocation and/or laceration; there is also a reduction in fitness, being harder to hunt and easier to be hunted (Laist, 1987; Derraik, 2002; Gall *et al*. 2015).

Chemical effects

Even though the most known effects of plastic ingestion are the physical effects, there are also chemical effects related to plastic ingestion, and those prove that plastic has chemical effects on the animals which ingested it and on the rest of the food chain (Teuten *et al*. 2007).

Some chemicals are POPs which are toxic, resistant, biocumulative, hydrophobic and easy to transport (Zarfl *et al*. 2010). Microplastics easily transport POPs so plastics with this contaminants can be found all over the world including on the Portuguese coast where some POPs have been reported (Frias *et al*. 2010). These chemicals may end up being ingested by marine animals. Surveys (Ryan, 1988; Yamashita *et al.* 2011) show that there is a direct relation between plastic ingestion and POPs presence in animals, and that the birds' guts conditions can facilitate the dissociation of POPs from plastics easier than in the marine environment (Bakir *et al*. 2014; GESAMP 2015). POPs' concentration may cause health issues as endocrine unregulation, teragonicity, hepatotoxicity and toxicity at kidney (Muirhead, 2006; Yogui *et al*. 2009). Additives are chemicals intentionally added to plastics during their manufacture or processing to be more effective at their purpose. Microplastics derived from compounded plastics may contain additives such as stabilizers, plasticizers or flame retardants. The effects of additives are similar to POPs effects, but studies defend that some additives induce behavioural changes in some species (Barse *et al*. 2007; Oehlmann *et al.* 2009).

Microplastics contaminated with chemicals after being ingested can be transferred in the trophic chain reaching the top predators. This is a major concern because many of these contaminated animals are consumed by humans, so this means that it is very important to study chemical effects of microplastic and the bioavailability of these chemicals.

In conclusion, the plastic can interact with organisms by different ways when it is incorporated by the organism. After incorporated, it can be excreted, bioaccumulated, bioconcentrated or continue at the trophic chain when the animal that first consumed it be himself ingested by another animal. At this stage it can be excreted by this new animal or continue into the trophic chain and be biomagnified (Provencher *et al*. 2019)(Fig. 4).

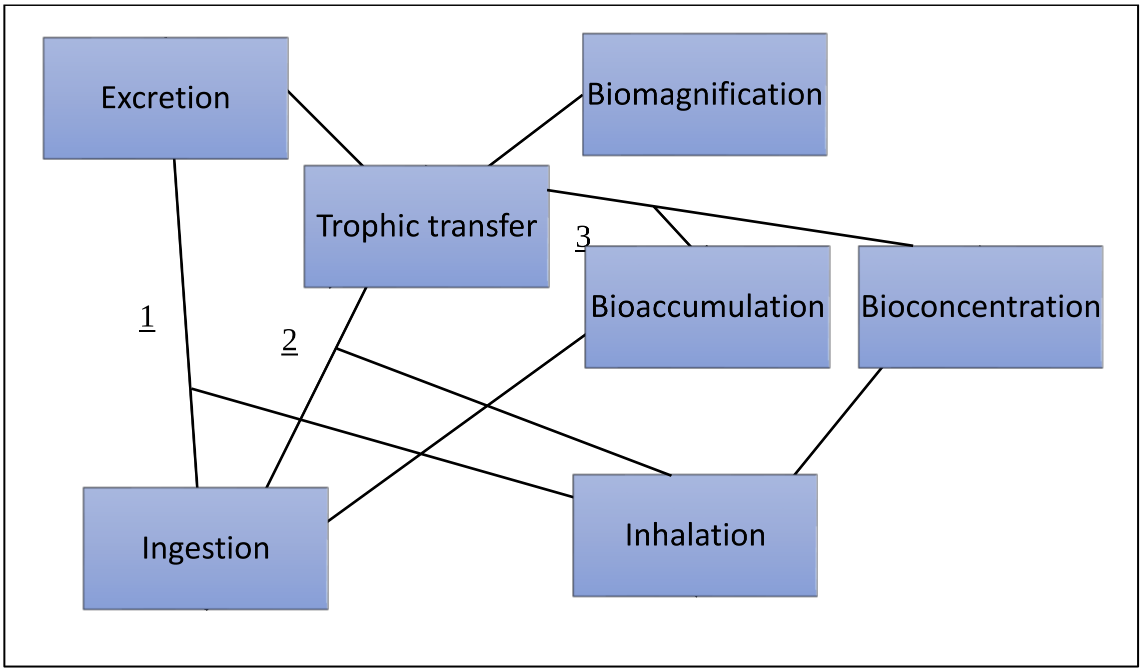

Figure 4. Pathways of microplastics in organisms. 1-Ingestion and inhalation can lead directly to excretion. 2-Ingestion and inhalation can lead to trophic transfer. 3-Microplastics can reach blood or tissues and via bioconcentration or via bioaccumulation be transferred at the food chain. Based on Provencher *et al*. 2019.

1.4 Seabirds as sentinels of plastic pollution

More than half of the world's seabird species have already been reported as plastic consumers (Roman *et al*. 2019). The quantity of consumed plastic varies mostly according to different diets and foraging methods of each species. Seabirds are reported to suffer from entanglement and ingestion of microplastic since the 1960s (Ryan *et al*. 2009). Some groups that suffer the most with this increasing concern are the fulmar (*Fulmarus glacialis*) and different species of shearwaters (Roman *et al*. 2019). Other species that suffers a lot is the Little auk (*Alle alle*) (Avery-Gomm *et al*. 2013), this species is zooplanktivorous so it may mistake their diet with microplastic.

Roman *et al*. 2016, in a survey in Australia, collected dead birds to study the presence of human litter in their guts: 30% of the birds had microplastic, including birds from all types of habitats and foraging techniques (surface feeding, pursuit diving and search-by-sight) but this percentage may be an underestimate because birds tend to regurgitate, so not all the ingested plastic will be found in the necropsy. The conclusion of this survey is that habitat is a very important variable, although species from all habitats are known to ingest plastic. The ingested plastic colours varied because of the feeding strategy or even because of colour preference by the birds (Verlis *et al*. 2013).

The risk of wedge-tailed shearwater (*Ardenna pacifica*) (WTS) to ingest microplastic was very high and this is due to some factors. WTS are procellariiformes and this order is known to have a high incidence of plastic interactions (Colabuono *et al*. 2009) because this seabird species may confuse plastics for prey as they follow boats in order to get feed easily. Another reason is that they feed by shallow plunging, contact dip, surface feeding and pursuit-plunge (Harrison *et al*. 1983). It was normal to expect that this species would consume a lot of debris, but Ainley *et al*. 1990 reported that this species ingest less plastic than other species, possibly because they pursuit active prey. Other important organisms in this study are the albatrosses (*Diomedeidae*) and petrels (*Procellariidae* and *Pelecanoididae*). These species forage far from the coast, at the South Atlantic Ocean, where there is a large concentration of plastic (Fig. 2). The conclusion of this study was that birds do not ingest plastics randomly, there are a lot of important variables, such as habitat, feeding behavior and plastic colour, that will influence plastic consumption by seabirds. Seabirds are very useful indicators of plastic pollution because they cover a wide area, however such wide covered area is also a negative aspect because it is very hard to know the exact location where seabirds feed, and to integrate this information in trophic transfer.

As plastic pollution is the nowadays "hot topic" several studies are published, though these studies were focused on specific regions, meaning that the plastic research is more intense in certain regions. The top regions for reports of ingested plastics in 2016 were the Eastern Central Pacific, the Southwest Atlantic, the Mediterranean and the North sea, where plastic ingestion work is mandatory, enabling comparisons between these studied regions (Provencher *et al*. 2019). Research in some other regions as temperate and sub-tropical North Atlantic is scarce, these two areas include Portugal and the African west coast. The comparison between these two regions is important because there are strong differences in the debris management between both areas. Portugal, a larger and more coastal populous country, has less plastic availability than Cabo Verde (Fig. 2). This is justified by the lack of waste management in the African continent (Worm *et al*. 2017).

The main goal of this project was to evaluate the role of seabird species in plastic ingestion, considering their feeding ecology and distribution. The main objective is divided in four secondary objectives. One of the objectives was to: (1) assess if the foraging ecology and the interaction with fisheries of each seabird species determines the amount of plastics ingested. We expect that coastal seabirds ingest more plastic than pelagic species, and also that the more generalist and obligate fisheries follower's species ingest more plastic than specialist species. Other objective was to (2) analyse if the location determines the ingestion of plastic, expecting that seabirds from the tropical area (Cabo Verde) present a higher amount of plastics than temperate (Portugal) seabird species. Other goal of this project was to (3) study the influence of the taxanomy and seabird size in plastic ingestion, with the expectation that larger species present larger plastic particles and that Procellariformes present more plastic than Suliformes. The last objective was to (4) analyse whether the interaction with fisheries determines the colour of plastics ingested by the seabirds, with the expectation that Audouin´s gull (*Ichthyaetus audouinii*) and *Calonectris sp.* present more green, blue and transparent plastics once they are species that interact with fisheries.

CHAPTER II - Materials & Methods

2.1 Study species

The study species included in this project are: Audouin´s gull (*Ichthyaetus audouinii*), Cory's shearwater (*Calonectris borealis*), Cape Verde Shearwater (*Calonectris edwardsii*), Red-billed Tropicbird (*Phaethon aethereus*), Brown booby (*Sula leucogaster*), Bulwer´s Petrel (*Bulweria bulwerii*) and Boyd´s Shearwater (*Puffinus lherminieri boydi*) (Fig. 5).

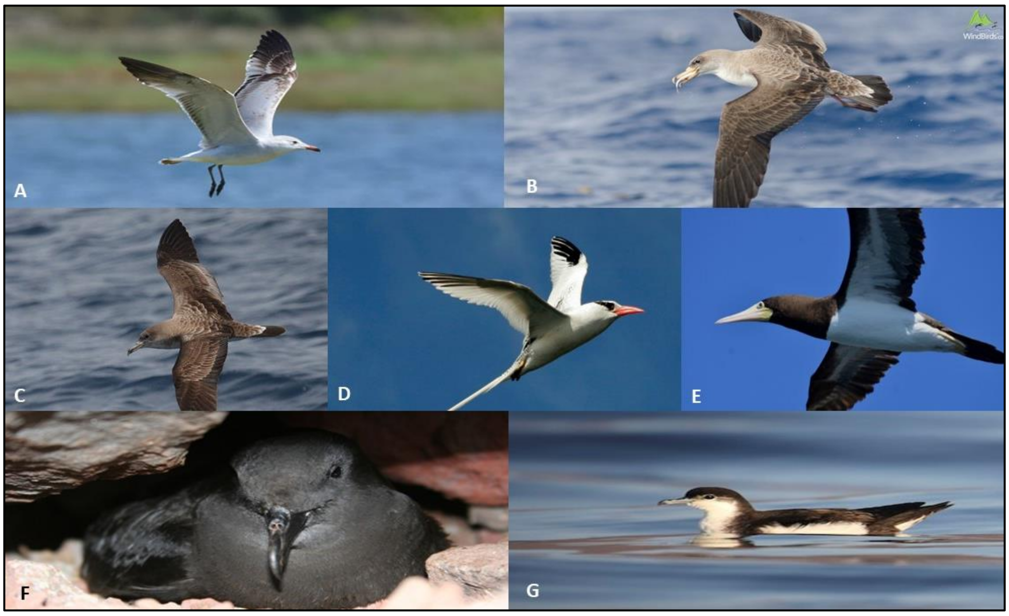

Figure 5. Study species. A- *Ichthyaetus audouinii* B- *Calonectris borealis* C- *Calonectris edwardsii* D- *Phaethon aethereus* E- *Sula leucogaster* F- *Bulweria bulwerii* G- *Puffinus lherminieri boydi.*

The Audouin's gull (*Ichthyaetus audouinii*) is a medium sized seabird with an average weight of 570g. Audouin´s gull (IA) is a seabird from *Laridae* family and is categorized as vulnerable on the International Unit for Conservation of Nature (IUCN) Red List (BirdLife International, 2021). The Audouin´s gull is a generalist forager mainly on the coastal area, feeding on small pelagic fish, but they also feed from trawler fisheries discards (Oro, 1995; Manosa *et al*. 2004). In Portugal it breeds only on the barrier Islands of Ria Formosa Natural Park, Algarve.

The Cory's shearwater (*Calonectris borealis*) is a medium sized seabird with an average weight of 850g (Paiva *et al*. 2009). Cory's shearwater (CB) is a seabird from *Procellariidae* family and is categorized as Least Concern on the IUCN Red List (BirdLife International, 2018b) and in mainland Portugal this species breeds mainly on Berlenga

Island (Lecoq *et al*. 2011). The species forages in both coastal and pelagic areas and mainly feeds on pelagic fish, with the less frequent consumption of cephalopod and crustacea prey (Paiva *et al*. 2010).

The Cape Verde Shearwater (*Calonectris edwardsii*) is a medium sized seabird with an average weight of 500 g (Semedo, 2020). The Cape Verde Shearwater (CE) is a seabird from the *Procellariidae* family and is categorized as near threatened on the IUCN Red List (BirdLife International, 2018c). The species forages in both coastal and pelagic areas and mainly feeds on pelagic fish, but they also feed on cephalopods and on fisheries subsidies (Aves Marinhas de Cabo Verde; Paiva *et al.* 2015).

The Red-billed Tropicbird (*Phaethon aethereus*) is a medium sized seabird with an average weight of 550 g (Semedo, 2020). The Red-billed Tropicbird (PA) is a seabird from *Phaethontidae* family and is categorized as Least concern on the IUCN Red List (BirdLife International, 2019). *Phaethon aethereus* forages between coastal and oceanic areas and mainly feeds on flying fish and squid (Aves Marinhas de Cabo Verde; Castillo-Guerrero *et al*. 2011).

The Brown booby (*Sula leucogaster*) is a large-sized seabird with an average weight of 1000 g (Semedo, 2020). The Brown booby (SL) is a seabird from the *Sulidae* family and is categorized as least concern on the IUCN Red List (BirdLife International, 2018e). The Brown booby forages almost exclusively in coastal areas, mainly feeding on pelagic fish (Aves Marinhas de Cabo Verde; Correia *et al.* 2021).

The Bulwer´s Petrel (*Bulweria bulwerii*), is a small seabird with an average weight of 100 g (Semedo, 2020). The Bulwer´s Petrel (BB) is a seabird from the *Procellariidae* family and is categorized as Least Concern on the IUCN Red List (BirdLife International, 2018a). The Bulwer´s Petrel is a highly specialized species, foraging in pelagic areas and feeding mainly on mesopelagic fish and squid (Aves Marinhas de Cabo Verde; Furtado *et al.* 2021).

The Boyd´s Shearwater (*Puffinus lherminieri boydi*) is a small sized seabird with an average weight of 160 g (Semedo, 2020). The Boyd´s Shearwater (PB) is a seabird from the *Procellariidae* family and is categorized as Least Concern on the IUCN Red List (BirdLife International, 2018d). The species forages in both coastal and pelagic areas, feeding mainly on pelagic fish (Aves Marinhas de Cabo Verde; Semedo, 2020; Santos *et al.* 2022).

Table 2. Study species and relevant information about their size, distribution, foraging habitat, diet and interaction with fisheries.

Species	*Size*	*Region*	*Foraging habitat*	*Diet*	*Fisheries interaction*
Ichthyaetus audouinii	Medium	Temperate	Coastal	Pelagic Fish, Scaveng ing	Yes
Calonectris borealis	Medium	Temperate	Coastal and pelagic	Pelagic Fish, squid	Yes
Calonectris edwardsii	Medium	Tropical	Coastal and pelagic	Pelagic Fish, squid	Yes
Phaethon aethereus	Medium	Tropical	Coastal	Flying fish	No
Sula leucogaster	Large	Tropical	Coastal	Pelagic Fish	No
Bulweria bulwerii	Small	Tropical	Pelagic	Mesopel agic Fish	No
Puffinus lherminieri boydi	Small	Tropical	Pelagic	Pelagic fish	No

2.2. Study area

Faecal samples from seabirds were collected between February and September 2021 in three sites, located on tropical (Raso Islet, Cabo Verde) (Fig. 6) and temperate (Desertas and Berlengas islands) (Fig. 7) marine environments.

Raso islet (16º 36’ 59’’N, 24º 35’ 21’’W) lays within the Cabo Verde archipelago located in the Atlantic Ocean, 385 km away from the African coast (Semedo, 2020). The island is a Natural Reserve since 1990 and a Marine Protected Area since 2003. This islet is uninhabited and is the habitat of several threatened and endemic species, though it is a relatively unknown area due to the islet remoteness and logistical situation (Pinho *et al. 2018)*. One of the main economic activities of Cabo Verde is coastal fishing (Monteiro *et al*. 2020).

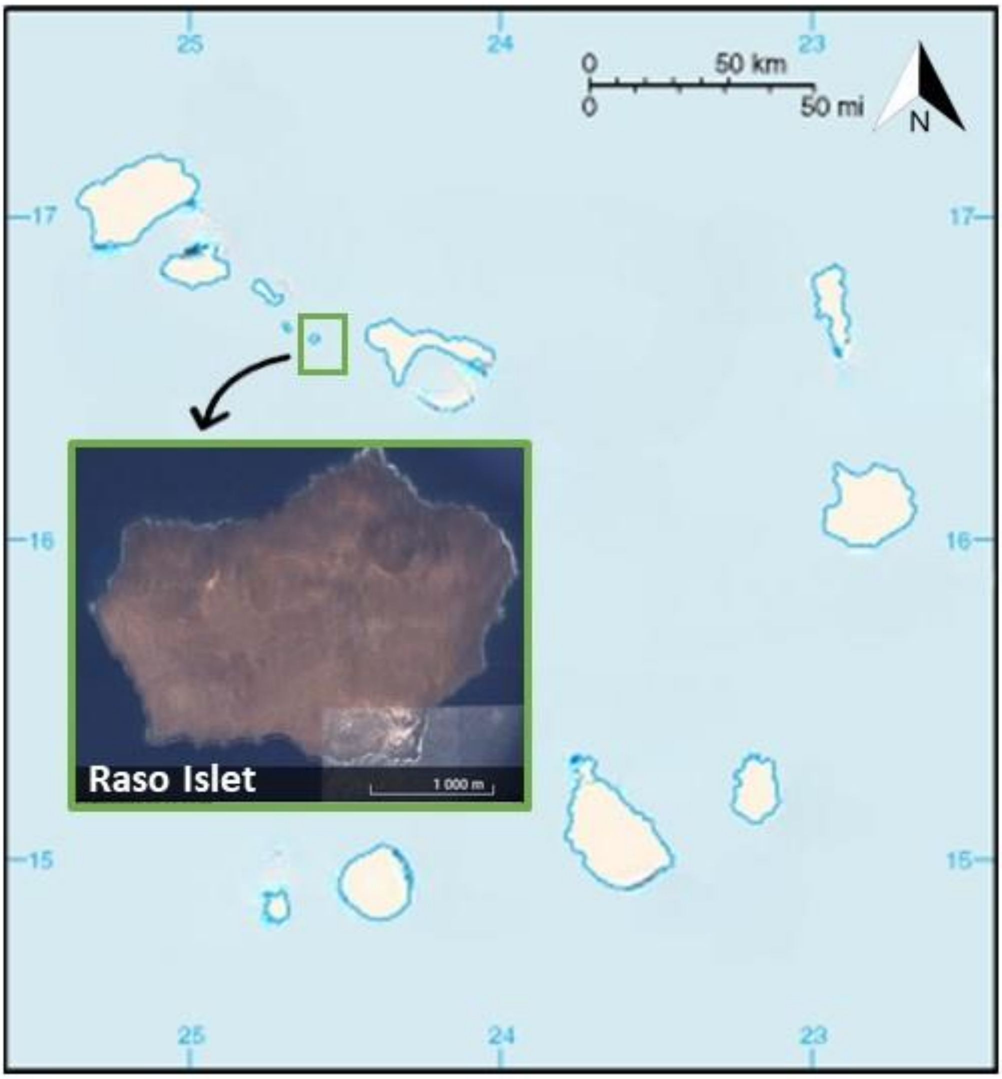

Figure 6. Geographical location of Raso Islet, (outlined in green) with aerial images taken from Google Earth.

Deserta Island (36º 57’ 53’’N, 7º 53’ 21’’W) (Fig.7), a non-habited sand-barrier Island 5.5 km distant from mainland Portugal, belongs to the Ria Formosa barrier island system located in Southern Portugal. This barrier island system is a notably important natural resource and provides activities such as nature conservation, tourism, ecotourism, aquaculture fisheries and effluent discharges, which are critical for the region and makes it an especially important environmental area. Ria Formosa is a Natural Reserve since 1978, a Natural Park since 1987, is integrated in the Natura 2000 project, and is also protected by the Ramsar and Bern conventions in order to make this ecosystem sustainable. This barrier island has an high piscatory activity possibly leading to fishing related debris (Ceia *et al*. 2010; Matos *et al*. 2018; Lopes *et al*. 2020, 2021).

Berlenga Island (39º 24’ 53’’N, 9º 30’ 31’’W) (Fig. 7), located in the Portuguese west coast (Lecoq *et al*. 2011), is an important seabird breeding colony for mainland Portugal. It is located 11 km far from mainland and it has seasonal intense coastal upwelling. This island firstly became a natural reserve in 1991, then in 1998 it was classified as a Marine Reserve Area and in 2011 it was added to the Biosphere UNESCO Reserve list (Mouga *et al*. 2021). It is only inhabited by a small community of fishers, though, in the summer this island suffers a huge tourism activity that exposes this area to several quantities of human litter (Lopes *et al*. 2020, 2021)

Figure 7. Geographical location of Deserta Island and Berlenga Island, (outlined in blue) with aerial images taken from Google Earth.

2.3 Sampling collection

The method used in this project was the faecal sampling. All the samples (n=344) were collected in the field using the same method, consisting first in the capture of the individual at the nest (all procellariiformes) or using a walk in nest trap (for Audouini's gull) . After the bird capture, it was stimulated gently until it defecated into a tube placed at the entrance of the cloaca (Fig. 8). The sample was collected using a disinfected spatula to an sterile Eppendorf. This collection method enabled to reduce the external contamination of the feces. The Eppendorfs were identified with the important information (Fig. 9). These samples were then stored in the refrigerator.

Figure 8. Bird stimulation to collect the faecal sample.

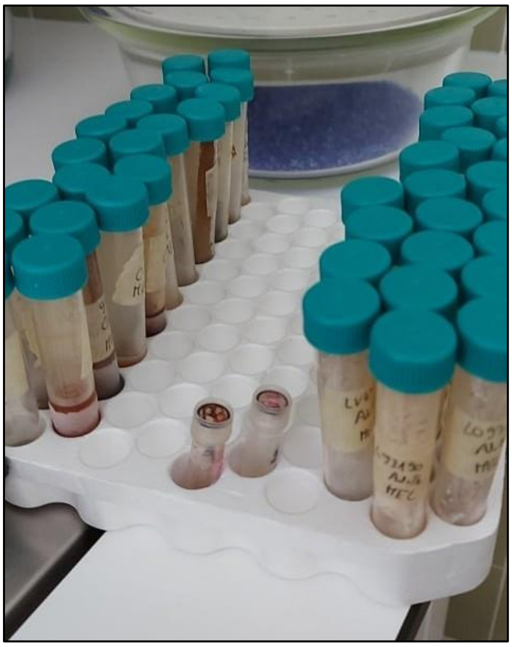

Figure 9. Identified Eppendorf with faeces.

2.4. Laboratorial procedures

In the laboratory the samples were stored in the refrigerator, so the first step of a sequential procedure (Fig. 10) was to defrost the samples and then dry them in the oven at 40ºC and during one day. The weight of every dry sample was noted.

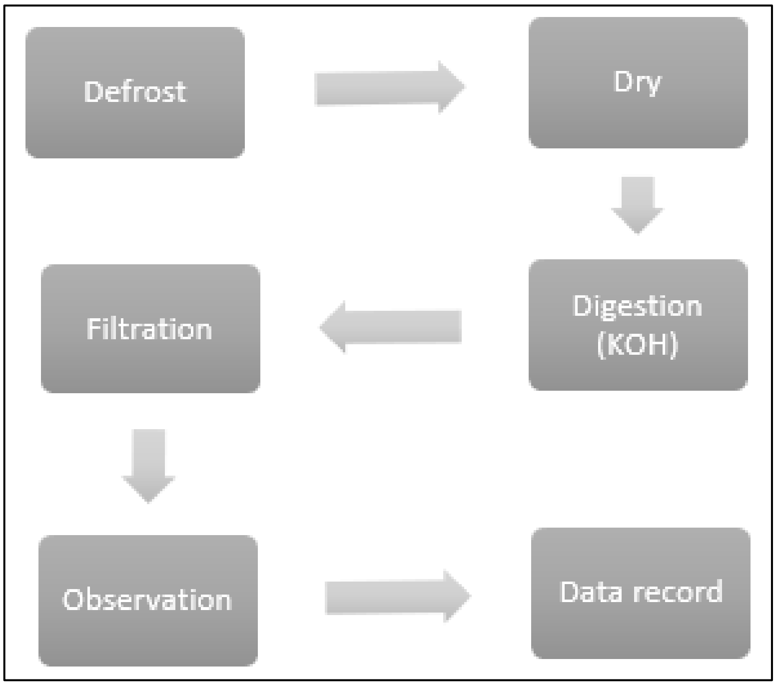

Figure 10. Workflow of the laboratorial procedures.

The digestion of the biological sample is crucial to remove the organic matter but simultaneously not degrading the, target of the study, plastics. Different approaches can be followed as Bessa *et al*. 2019 describes. At this point all the samples have the faecal material, including the organic matter and the plastic material (Fig. 11). As all the methods, the procedure used also has some disadvantages, the enzymatic digestion disadvantages are the microplastic damage due to the usage of strong oxidants (Nelms *et al*. 2018). The digestion used is the alcaline treatment, using KOH 10% (POHY P0A-5K0 from LABKEM) until full digestion occurs, during 24 hours at room temperature, the digestion period can vary according to the amount of organic matter in the sample.

The second step in the laboratory was to transfer the faecal material in the Eppendorf to a 250 ml glass beaker and fill the beaker with a volume of solution 3 times the volume of the sample (Fig. 12). The digestions had a medium duration of a day and half at room temperature.

Figure 11. Identified Eppendorfs and glass beakers.

Figure 12. Sample digestion in a beak.

After the digestion process the remaining solution is vacuum filtered (Fig. 13), using Branchia microfiber glass paper filter (47mm diameter, 1.2 µm grid). The filters were placed in Petri dishes properly identified and transferred to the oven at 40˚C for 48 hours. Hydrogen peroxide (H_2O_2 10%) was added to the filters with high organic matter quantity in order to clarify the sample and were placed again in the oven one hour to be totally dry.

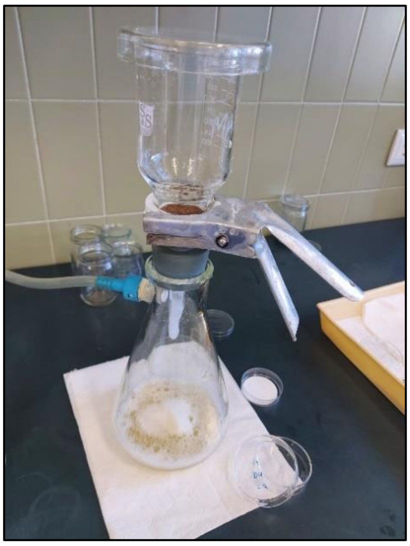

Figure 13. Filtration kit.

The filtration process occurred in a controlled access area in order to minimize the contamination probability (Fig. 14), and also, during the process, control filters (one inside and other outside the camera) were exposed to collect contamination, and changed every week. Other contamination free practices were the usage of sterilized glass laboratory material, cotton coats and nitrile gloves. All the liquids were filtered, and the containers were washed with alcohol and ultrapure water before reuse (Fig. 15).

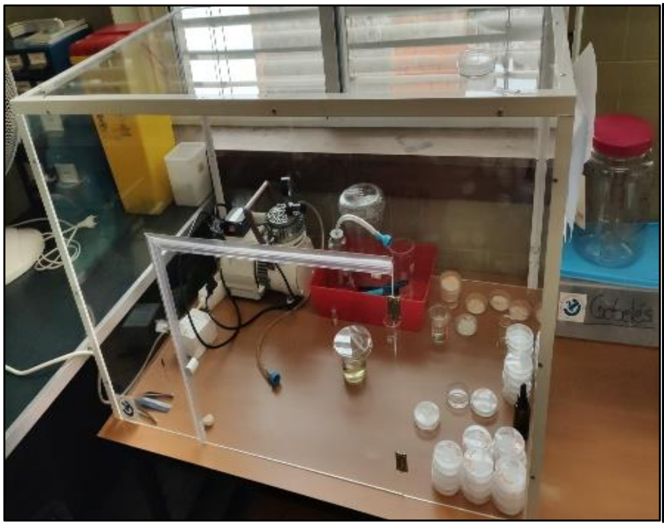

Figure 14. No air flux chamber.

Figure 15. Washing with ultrapure water.

All the filters obtained in the filtrations and also the contamination controls filters were observed and photographed using a stereomicroscope LEICA M80 (Fig. 16) (Leica Microsystems GmbH, Wetzlar, Germany) with image analysis system IC80 HD Camera with Leica Application Suite (LAS) software. The particles in the filtered filters are characterized as “potential plastic” (Fig. 17) since they do not degrade after the KOH treatment. These particles were classified and categorized by type into fragment, fiber, filament, microbead, rubber and ball. They were also classified by colour into black, blue, green, grey, orange, pink, purple, red, transparent, white, yellow and multicolour. Finally, they were measured using the longest cross section, and classified as mesoplastics (5mm to 2.5cm), large microplastics (1mm to 5mm), small microplastics (1µm to 1mm) and nanoplastics (1nm to 1µm).

Figure 16. Stereomicroscope Leica M80.
(http://irvinescientific-art-eu.fujifilm.com/pt)

To minimize any contamination, the entire process of microplastics identification was performed in the same conditions as in the sample processing.

All the laboratorial procedure is based in Bessa *et al.* 2018, 2019 and Frias, 2018.

Figure 17. Photograph of particles found in faeces samples. A- Fiber B- Fragment C-Filament.

2.5. Data analysis

A table including all samples with/without the presence of plastics, with the number of plastics per affected bird of each species, the mean plastic size and the mean sample weight of samples with plastics was constructed using Excel (Table 3). In five cases the samples were not weighted before analysed, and the median sample weight of the species was used for these samples. Fifty sizes of plastics were not measured and, in order to calculate the average plastic size, the mode value was used instead. The mean number of plastics was computed just with the individuals containing at least a piece of plastic. Normal distribution was not possible to achieve even after transformations (log normal, log10 and square root transformations, with Shapiro-Wilk test <0.05).

Binomial matrixes of presence/absence for the categories plastic type (fiber, fragment, filament, microbead, rubber and ball), colour (blue, purple, transparent, grey, pink, orange and multicolour) and size (mesoplastic, large microplastic, small microplastic and nanoplastic) were constructed in excel for each species individually, region (temperate, tropical), distributions (coastal, pelagic), sizes (small from 100g to 160g, medium from 500g to 850g and large with 1000g) and fisheries interaction (with and without). The frequency of occurrence of each category (F.O.) was calculated using the formula: $FO_x = \frac{n_x}{n_{total}} * 100$, where x represents the category, n_x is the number of affected samples of the x category and n_{total} corresponds to the total number of analysed samples of each class. Tables with the results (Table 6, 10, 12, 14 and 16) and respective pie charts (Fig. 18) were constructed using Excel.

Generalized linear model (GLM) with the binomial distribution and logit link function were made to analyse the presence or absence of plastics in the species from different regions, distributions, sizes, fisheries interaction and also in all the species individually (Table 4 and 7). All this GLM tested the effect on the following response variables: presence/absence, plastic type, plastic colour and plastic size with a significance level of $p < 0.05$. Only the classes with an overall occurrence above 10% were used for the statistical analysis.

Matrixes of numerical frequency were constructed using Excel. The frequency of plastics of each category was calculated using the formula $FN_x = \frac{n_x}{n_{total}} * 100$,where x represents the category, n_x is the number of plastics of the x category and n_{total} corresponds to the total number of plastics of each class. Tables with the results (Table 8, 11, 13, 15 and 17) and respective bar charts (Fig. 22, 23, 24 and 25) and pie charts (Fig. 26) were constructed using Excel.

The effect of species, region, size, distribution and interaction with fisheries was tested using a generalized linear model with a Poisson distribution and a significance level of $p < 0.05$. Zero inflaction fitting and zero inflated with negative binomial distribution fitting were performed. The best fitting models were selected based on the lowest Akaike's Information Criterion (AIC) and log-likelihood. Only classes with an overall occurrence above 10% were used (Table 5 and 9).

Non-metric multidimensional scaling (NMDS) using the number of plastics of each category per sample was used to graphically represent the dissimilarities between the species in each category (type, colour and size) (Fig. 19, 20 and 21).

In order to analyse possible correlation between weight of the faecal sample and the number of microplastics, a Spearman´s rank correlation coefficient was calculated. This nonparametric test was made in Statistica version 12.

The R statistical program (4.2.0), using the Rstudio-2022.02.1 Build 461 interface, was used to perform GLM and NMDS. GLM were performed using the integrated statistic package of R, and also "performance" and "pscl" R packages (Zeileis *et al.* 2008; Jackman, 2017). NMDS were done using "Vegan", "Dplyr","Viridis" and "Ggplot2" R packages (Oksanen *et al.* 2019; Bates *et al.* 2022).

CHAPTER III – Results

3.1 Presence of plastics in the faeces of Seabirds from Portugal and Cabo Verde

A total of 344 faecal samples were collected which contained 388 potential plastic items. Plastics were detected in all study species, with *Bulweria bulwerii* (BB; 35.9%) and *Calonectris borealis* (CB; 41.9%) exhibiting the lowest frequency of occurrence (F.O.) of plastics and *Calonectris edwardsii* (CE; 86.5%) exhibiting the highest F.O.. CE (2.4 ± 1.60 items ind.$^{-1}$) and *Phaethon aethereus* (PA; 2.4 ± 1.47 items ind.$^{-1}$) showed the higher mean number of plastics per individual bird. Plastics of larger dimension were detected in *Ichthyaetus audouinii* (IA; 1.8 ± 1.21 mm) and the species that presented plastics with smaller dimensions was CE (0.5 ± 0.95 mm) (Table 3).

Table 3. Comparison of the presence of plastics, number of plastic items, mean number of plastics per affected bird, mean size of plastics and mean sample weight of samples with plastics among all the studied species. Standard deviation represented as ± SD.

Species	N	Presence%	Number of plastic items	Mean number of plastics per affected bird ± SD	Mean size of plastics (mm) ± SD	Mean weight (mg) of samples with plastics ± SD
Ichthyaetus audouinii	32	62.5	39	2.0 ± 1.24	1.8 ± 1.21	2.2 ± 0.46
Calonectris borealis	43	41.9	25	1.4 ± 0.76	1.1 ± 1.06	2.2 ± 1.67
Calonectris edwardsii	52	86.5	110	2.4 ± 1.60	0.5 ± 0.95	0.5 ± 0.37
Phaethon aethereus	34	64.7	53	2.4 ± 1.47	0.7 ± 1.20	0.2 ± 1.15
Sula leucogaster	54	68.5	85	2.3 ± 1.50	1.1 ± 1.16	0.5 ± 0.41
Bulweria bulwerii	39	35.9	21	1.3 ± 0.82	0.6 ± 1.87	0.1 ± 0.06
Puffinus lherminieri boydi	90	44.4	55	1.4 ± 0.73	0.7 ± 1.00	0.1 ± 0.11

Fragments and fibers were the plastic types most common in all the species. In terms of size, the smallest and largest sizes of microplastics were the most common in all species. In terms of colour, blue was the most common in the faecal samples (Fig. 18 and table 6).

When comparing the frequency of occurrence among all species (table 4 and 7), and considering IA the reference species, only CE differed significantly, as it presented more plastics than the reference species.

Regarding types, the presence of fragments differed significantly in CE, PA and *Sula leucogaster* (SL), because the presence of fragments was higher in these species than in the reference species. In terms of fibers BB and *Puffinus lherminieri boydi* (PB) showed a significantly lower presence than IA.

In terms of colours, CE presented a higher presence of blue plastics than the reference species, contrary to PB that had a significantly lower presence of blue than the reference species. CB, SL and PB had significantly less samples with transparent plastics than IA. Orange was significantly less present in CE and PA than in IA.

CE had a higher number of samples with presence of mesoplastics than the reference species. Large microplastics had a significantly lower presence in CB, PA, BB and PB than in IA. Small microplastics had a significantly higher presence in CE, PA and SL, than in IA.

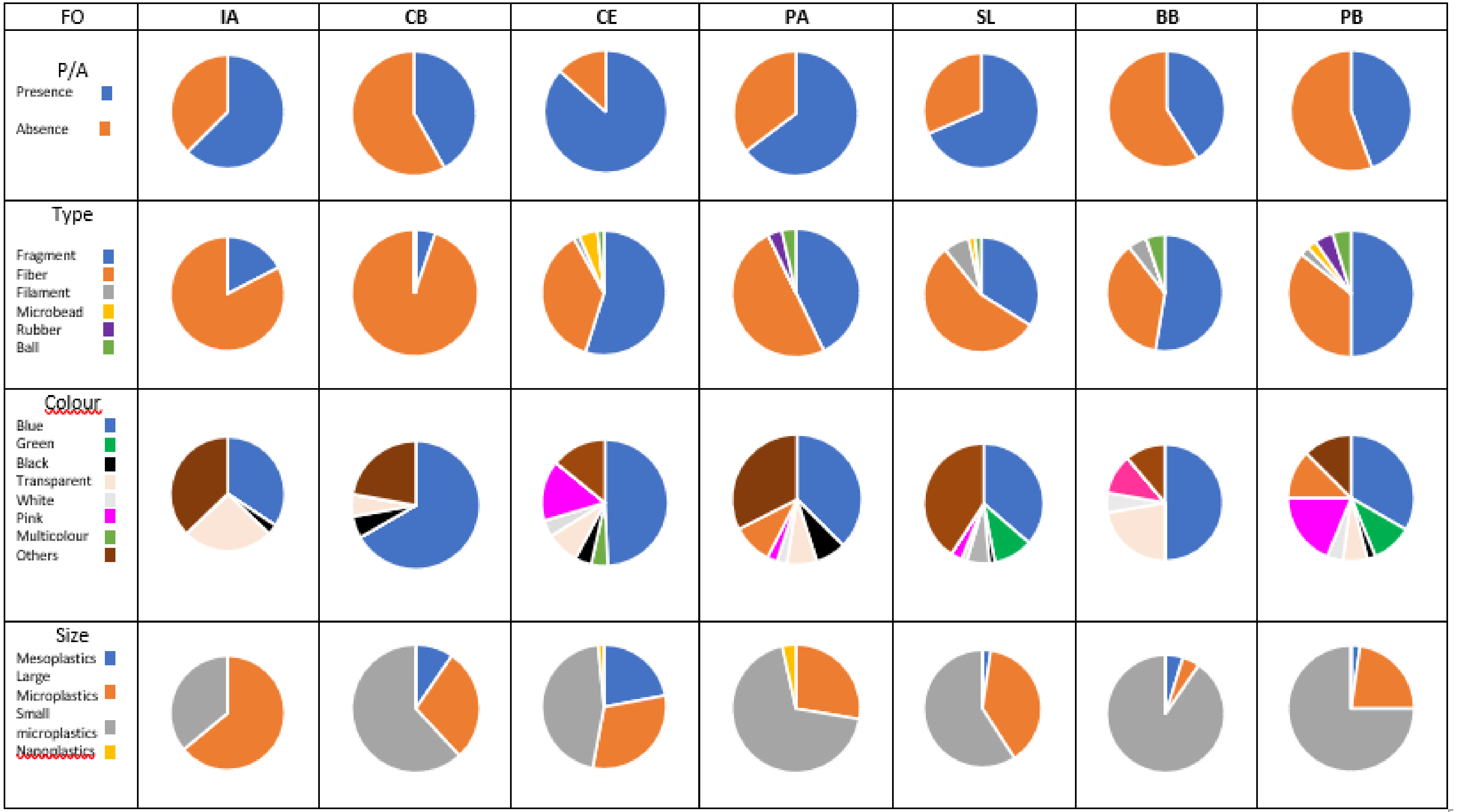

Figure 18. Frequency of occurrence of the plastics' presence/ absence, type, colour and size (mesoplastics (5mm to 2.5cm), large microplastics (1mm to 5mm), small microplastics (1µm to 1mm) and nanoplastics (1nm to 1µm)) in *Ichthyaetus audouinii* (IA; N=32), *Calonectris borealis* (CB; N =43), *Calonectris edwardsii* (CE; N =52), *Phaethon aethereus* (PA; N =34), *Sula leucogaster* (SL; N =54), *Bulweria bulwerii* (BB; N =39), *Puffinus lherminieri boydi* (PB; N =90).

Table 4. Generalized linear model testing the effect of seabird species on the plastics' presence, type, colour and size (mesoplastics (MP; 5mm to 2.5cm), large microplastics (LMP; 1mm to 5mm), small microplastics (SMP; 1µm to 1mm) and nanoplastics (NP; 1nm to 1µm)). Significant results are in **bold.** Models with non-significant results are not shown on the table, namely filament (p > 0.995), microbead (p > 0.997), rubber (p > 0.998), ball (p > 0.998), purple (p > 0.989), grey (p > 0.992) pink (p > 0.992) and multicolour (p > 0.997).

Species	Count model	Presence	Fragment	Fiber	Blue	Transparent	Orange	MP	LMP	SMP
Calonectris borealis	β ± SE	-0.712 ± 0.484	-1.718 ± 1.145	-0.580 ± 0.480	-0.337 ± 0.502	-2.725 ± 1.086	-19.628 ± 2803.418	0.490 ± 1.248	-1.735e+00 ±5.666e-01	0.207 ± 0.518
	Z	1.470	1.500	1.208	0.670	2.509	0.007	0.392	3.061	0.400
	P	0.142	0.134	0.227	0.503	**0.012**	0.994	0.695	**0.002**	0.689
Calonectris edwardsii	β ± SE	1.350 ± 0.546	2.582 ± 0.609	-0.611 ± 0.456	1.414 ± 0.477	-1.099 ± 0.586	-2.994 ± 1.084	2.531 ± 1.061	-3.895e-01 ±4.526e-01	1.328 ± 0.484
	Z	2.471	4.241	1.342	2.967	1.876	2.763	2.385	0.860	2.742
	P	**0.014**	**<0.001**	0.180	**0.003**	0.061	**0.006**	**0.017**	0.390	**0.006**
Phaethon aethereus	β ± SE	0.095 ± 0.512	1.340 ± 0.644	-0.736 ± 0.501	0.274 ± 0.503	-1.397 ± 0.721	-2.558 ± 1.089	-15.132 ± 1118.624	-1.179e+00 ±5.371e-01	1.295 ± 0.525
	Z	0.186	2.081	1.469	0.546	1.937	2.350	0.014	2.195	2.465
	P	0.852	**0.037**	0.142	0.585	0.053	**0.019**	0.989	**0.028**	**0.014**
Sula leucogaster	β ± SE	0.267 ± 0.468	1.335 ± 0.606	-0.081 ± 0.453	0.288 ± 0.456	-1.588 ± 0.652	-1.141 ± 0.585	-0.536 ± 1.432	-7.777e-01 ±4.592e-01	1.313 ± 0.481
	Z	0.570	2.204	0.179	0.630	2.436	1.951	0.374	1.694	2.730
	P	0.569	**0.028**	0.858	0.529	**0.015**	0.051	0.708	0.090	**0.006**
Bulweria bulwerii	β ± SE	-0.874 ± 0.489	0.881 ± 0.648	-2.549 ± 0.639	-0.693 ± 0.527	-1.231 ± 0.658	-19.628 ± 2839.131	-0.204 ± 1.435	-3.638e+00 ±1.073e+00	0.127 ± 0.524
	Z	1.786	1.359	3.989	1.315	1.870	0.007	0.142	3.390	0.243
	P	0.074	0.174	**<0.001**	0.188	0.061	0.994	0.887	**<0.001**	0.808
Puffinus lherminieri boydi	β ± SE	-0.734 ± 0.422	0.756 ± 0.590	-1.989 ± 0.458	-1.021 ± 0.458	-2.429 ± 0.707	-19.628 ± 1868.945	-1.055 ± 1.430	-2.079e+00 ±4.873e-01	0.392 ± 0.449
	Z	1.738	1.282	4.345	2.231	3.437	0.011	0.738	4.267	0.871
	P	0.082	0.200	**<0.001**	**0.026**	**<0.001**	0.992	0.461	**<0.001**	0.384

Most of the plastics found in the faeces were fragments (N= 166) and fibers (N=200). In terms of size, large microplastics (N= 116) and small microplastics (N= 244) were the most common. Additionally, most of the plastics were blue (N= 192) (Table 8 and Fig. 26).

When assessing differences in the number of plastics among species most of the categories fitted better with a Poisson distribution, only “total”, “fiber” and “large microplastics” had a better fit with zero inflated models with a negative binomial distribution fitting (Table 5 and 9). The only species that presented significant differences in the total plastics number was PB since it has less plastics in the samples than the reference species. PA and SL presented less fragments while CE presented more fragments than the reference species.

Concerning colours, CE had a significantly higher number of blue plastics than the reference species. CB, PA, SL and PB showed significant differences in the category “transparent” and had less transparent particles when compared with the reference species IA**.** The species CE and PA presented significantly fewer orange plastics than the reference species.

CB had significantly less large microplastics than IA. Small microplastics presented significant values to CE and PA for having a higher quantity of small microplastics than the reference species.

Table 5. Generalized linear model testing the effect of seabirds species and plastics' number, type, colour and size (mesoplastics (MP; 5mm to 2.5cm), large microplastics (LMP; 1mm to 5mm), small microplastics (SMP; 1µm to 1mm) and nanoplastics (NP; 1nm to 1µm)) of numeric frequency data applying Poisson distribution, zero inflated model and zero inflated with negative binomial distribution model. The table presents the best fit model of each category. Significant results are in **bold**. Models with non-significant results are not shown on the table, namely fiber ($p > 0.071$), filament ($p > 0.997$), microbead ($p > 0.997$), rubber ($p > 0.998$), ball ($p > 0.995$), purple ($p > 0.991$) grey ($p > 0.995$), pink ($p > 0.991$), multicolour ($p > 0.997$) and mesoplastic ($p > 0.994$).

Species	Count model	N total	Fragment	Blue	Transparent	Orange	LMP	SMP
Calonectris borealis	β ± SE	-0.820 ± 0.457	-1.905 ± 1.095	-0.044 ± 0.356	-2.000 ± 0.769	-20.034 ± 3907.654	-1.512 ± 0.451	-0.072 ± 0.387
	Z	1.794	1.739	0.124	2.602	0.005	3.353	0.187
	P	0.073	0.082	0.901	**0.009**	0.996	**<0.001**	0.852
Calonectris edwardsii	β ± SE	0.440 ± 0.277	2.236 ± 0.462	1.019 ± 0.295	-0.938 ± 0.484	-2.683 ± 1.054	-0.318 ± 0.491	1.157 ± 0.315
	Z	1.588	4.843	3.447	1.939	2.545	0.648	3.668
	P	0.112	**<0.001**	**<0.001**	0.053	**0.011**	0.517	**<0.001**
Phaethon aethereus	β ± SE	0.351 ± 0.318	1.326 ± 0.500	0.558 ± 0.332	-1.360 ± 0.651	-2.259 ± 1.054	-0.193 ± 0.642	1.118 ± 0.330
	Z	1.106	2.651	1.685	2.088	2.142	0.301	3.387
	P	0.269	**0.008**	0.092	**0.037**	**0.032**	0.763	**<0.001**
Sula leucogaster	β ± SE	0.278 ± 0.294	1.086 ± 0.490	0.501 ± 0.312	-1.312 ± 0.539	-0.775 ± 0.504	-0.253 ± 0.510	1.035 ± 0.318
	Z	0.947	2.217	1.609	2.432	1.537	0.496	3.258
	P	0.344	**0.027**	0.108	**0.015**	0.124	0.620	**0.001**
Bulweria bulwerii	β ± SE	-0.586 ± 0.463	0.678 ± 0.532	-0.352 ± 0.393	-1.209 ± 0.584	-20.034 ± 4103.156	-3.494 ± 1.039	0.262 ± 0.369
	Z	1.266	1.273	0.895	2.071	0.005	3.361	0.710
	P	0.206	0.203	0.371	**0.038**	0.996	**<0.001**	0.478
Puffinus lherminieri boydi	β ± SE	-0.801 ± 0.290	0.652 ± 0.487	-0.677 ± 0.348	-2.333 ± 0.651	-20.034 ± 2701.028	-0.917 ± 0.733	0.195 ± 0.328
	Z	2.759	1.340	1.944	3.582	0.007	1.251	0.593
	P	**0.006**	0.180	0.052	**<0.001**	0.994	0.211	0.553

Non-metric multidimensional scaling (NMDS) did not present a clear separation among species in terms of plastic types (Fig. 19). In terms of colours it is possible to verify that IA and SL are separated from the other species by the NMDS2 (Fig. 20). In terms of plastic size, the NMDS 1 separated BB from IA, from the rest of the species (Fig. 21).

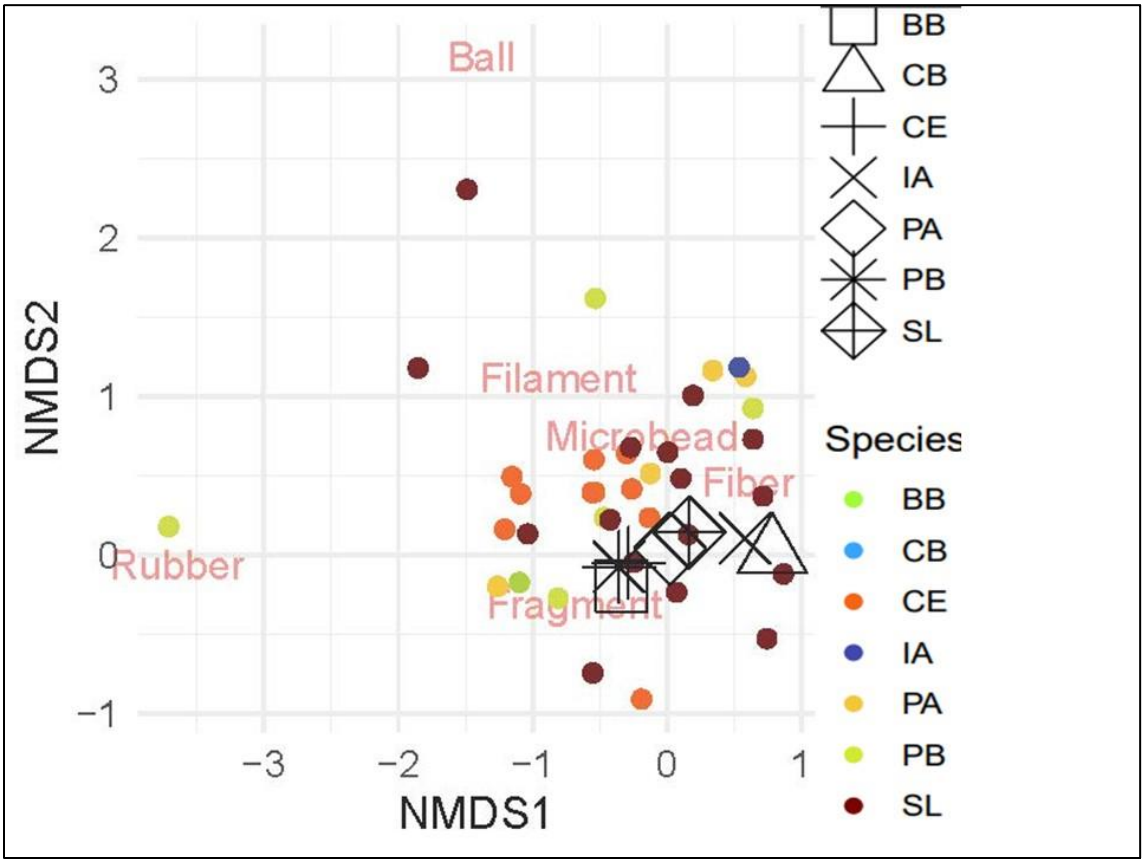

Figure 19. Non-metric multidimensional scaling (NMDS) using the numeric frequency of plastics' types. *Ichthyaetus audouinii* (IA), *Calonectris borealis* (CB), *Calonectris edwardsii* (CE), *Phaethon aethereus* (PA), *Sula leucogaster* (SL), *Bulweria bulwerii* (BB), *Puffinus lherminieri boydi* (PB).

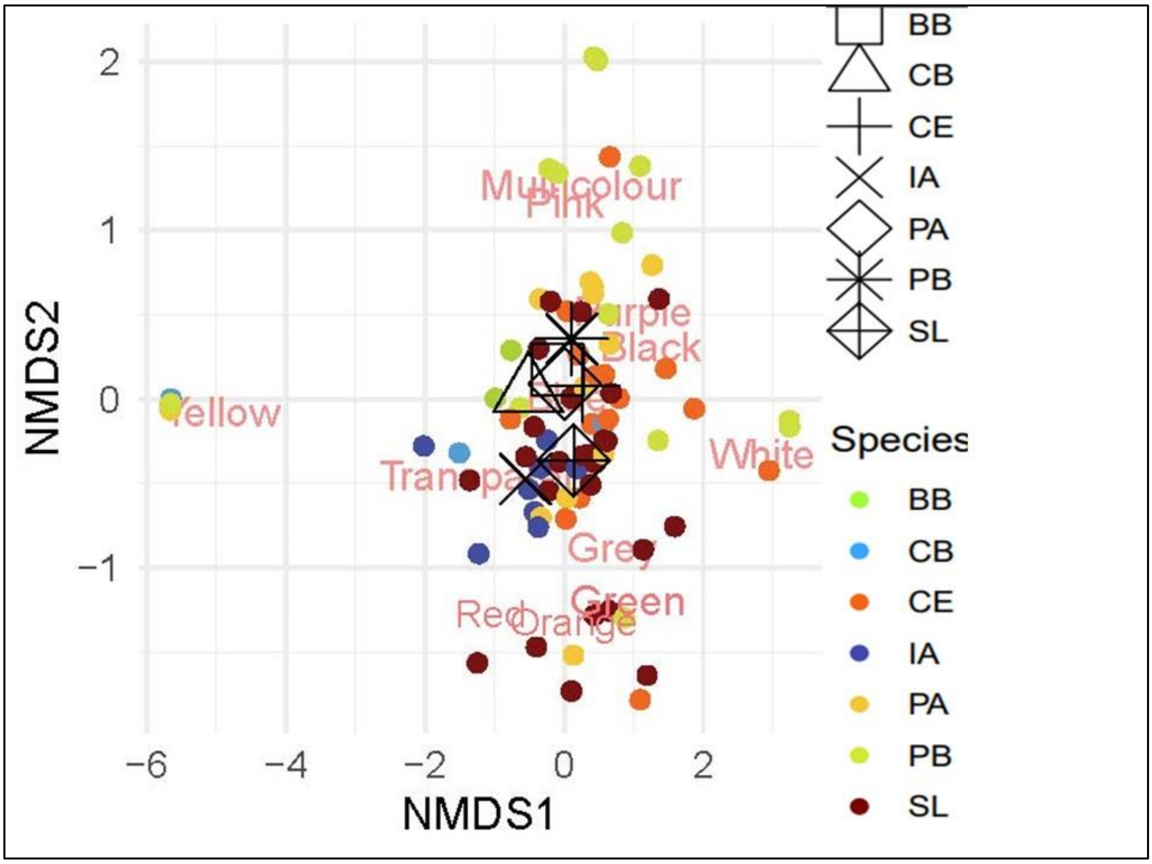

Figure 20. Non-metric multidimensional scaling (NMDS) using the numeric frequency of plastics' colours. *Ichthyaetus audouinii (*IA), *Calonectris borealis* (CB), *Calonectris edwardsii* (CE), *Phaethon aethereus* (PA), *Sula leucogaster* (SL), *Bulweria bulwerii* (BB), *Puffinus lherminieri boydi* (PB).

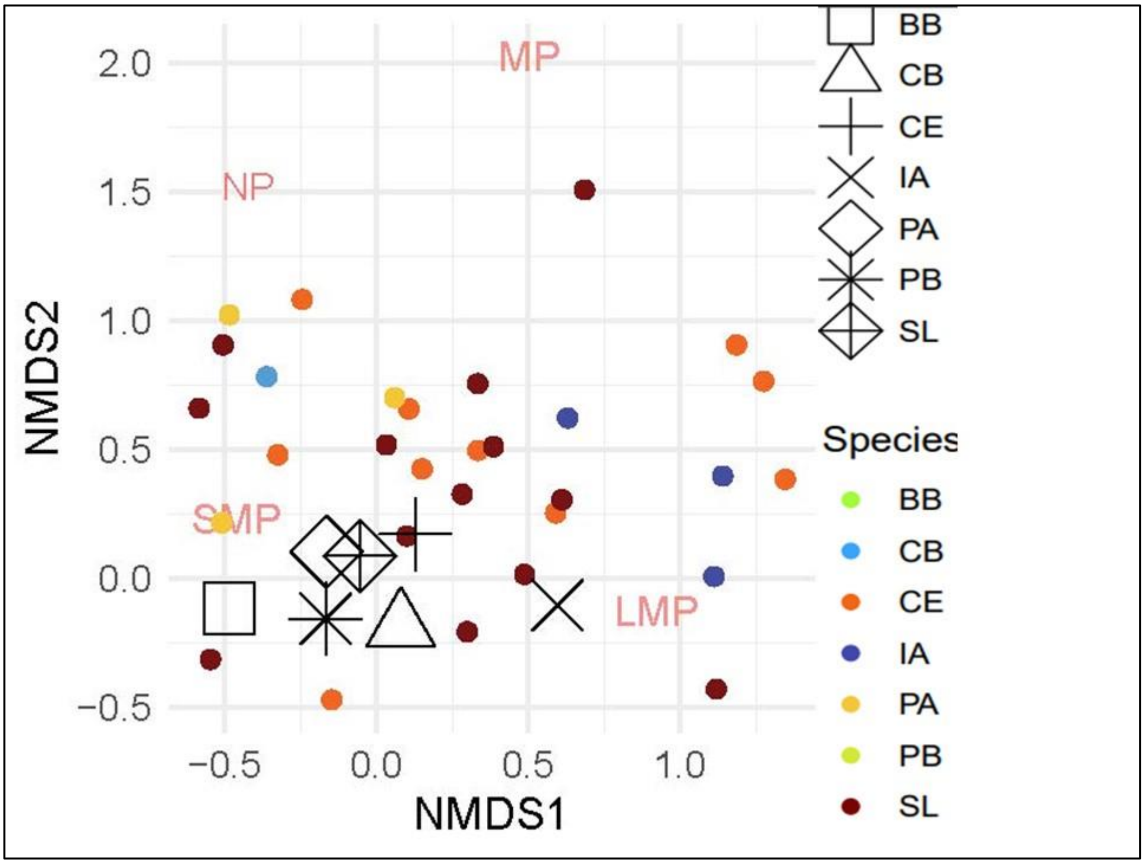

Figure 21. Non-metric multidimensional scaling (NMDS) using the numeric frequency of plastics' sizes. *Ichthyaetus audouinii* (IA), *Calonectris borealis* (CB), *Calonectris edwardsii* (CE), *Phaethon aethereus* (PA), *Sula leucogaster* (SL), *Bulweria bulwerii* (BB), *Puffinus lherminieri boydi* (PB). Mesoplastics (5mm to 2.5cm), large microplastics (1mm to 5mm), small microplastics (1µm to 1mm) and nanoplastics (1nm to 1µm).

Additionally, there was no correlation between the number of plastics and the weight of the samples (Spearman´s Rank correlation $R = 0.132$; $p = 0.066$; $N = 196$).

3.2 Analyses of the amount of plastics present in seabirds from different regions

Based on the data from the two different regions, temperate and tropical, it was possible to find differences in the occurrence of potential plastics (Table 10). In terms of presence there are no significant differences between both regions. In terms of the presence of fragments, seabirds from the tropical region ($\beta = 2.006 \pm 0.481$; $Z = 4.173$; $p < 0.001$) had significantly more plastics than temperate seabirds (the reference category). Samples from the tropical region ($\beta = -0.794 \pm 0.269$; $Z = 2.946$; $p = 0.003$) had a significantly lower occurrence of fibers than temperate seabirds, and a higher occurrence of samples with small microplastics ($\beta = 0.724 \pm 0.284$; $Z = 2.555$; $p = 0.011$) than temperate seabirds. In terms of colours, tropical seabirds had a lower occurrence of samples contaminated with orange plastics than temperate seabirds ($\beta = -1.539 \pm 0.506$; $Z = 3.043$; $p = 0.002$).

When assessing differences in the number of plastics among regions most of the categories fitted better with a Poisson distribution, but “total number”, “small microplastic” fitted better a zero inflated model, and number of “blue plastics”, “large microplastic”, “mesoplastic”, and “fibers” had a better fit with zero inflated models with a negative binomial distribution fitting. Tropical seabirds presented a significantly higher total number of plastics ($\beta = 0.345 \pm 0.171$; $Z = 2.021$; $p = 0.043$), as well as significantly more fragments ($\beta = 2.264 \pm 0.557$; $Z = 4.062$; $p < 0.001$) than temperate seabirds. In terms of number of plastics by size there were no significant differences between the two regions. Tropical seabirds had a significantly lower number of transparent plastics ($\beta = -0.751 \pm 0.350$; $Z = 2.147$; $p = 0.032$), as well as of orange plastics ($\beta = -1.277 \pm 0.471$; $Z = 2.709$; $p = 0.007$) than temperate seabirds (Table 11 and Fig. 22).

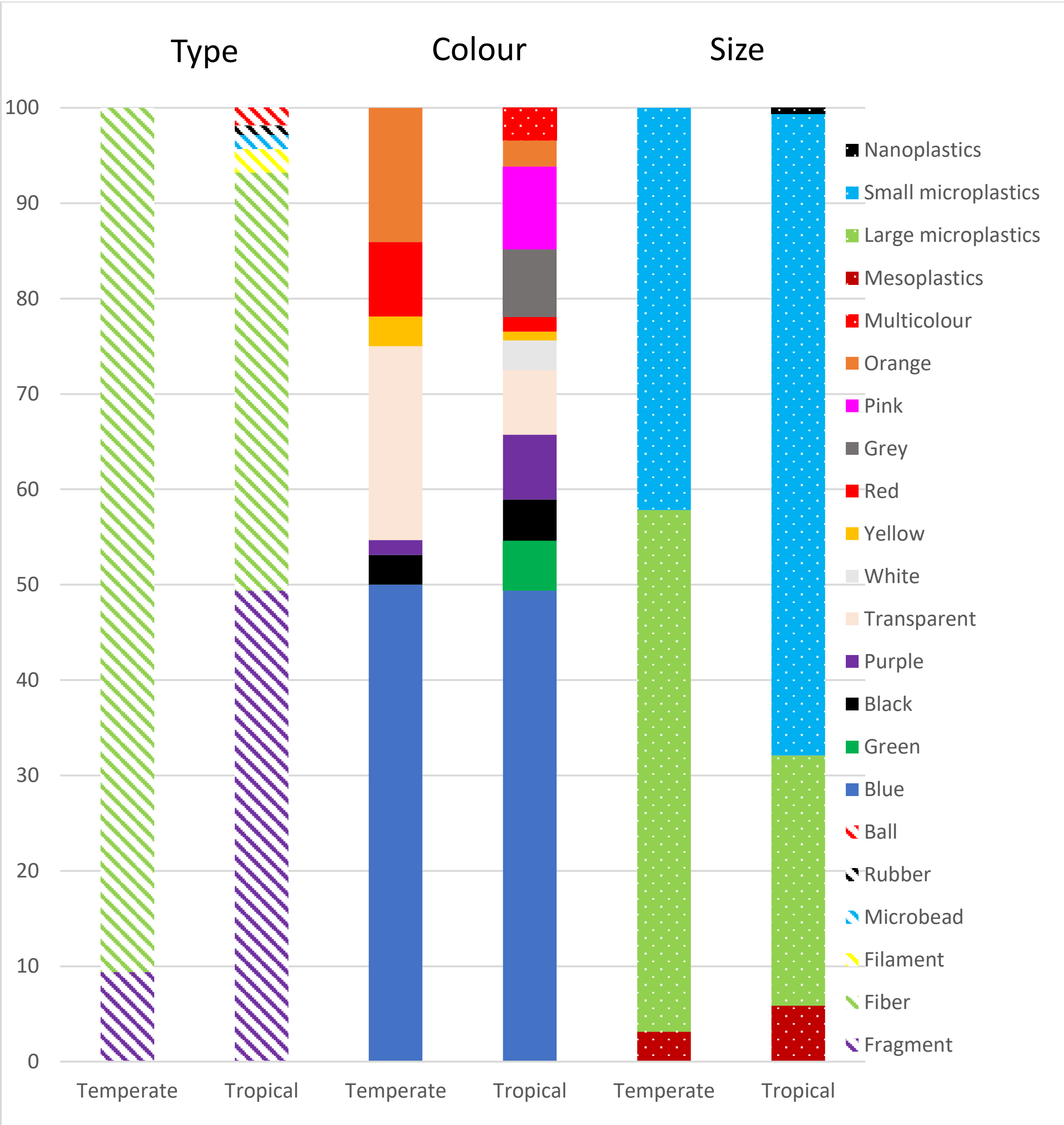

Figure 22. Numeric frequency bar chart comparing temperate (N = 75; number of plastics = 64) species: *Ichthyaehus audouinii* and *Calonectris borealis*, and tropical (N = 269; number of plastics = 324) species: *Calonectris edwardsii*, *Phaethon aethereus*, *Sula leucogaster*, *Bulweria bulwerii* and *Puffinus lherminierii boydi*. Sizes are divided into mesoplastics (5mm to 2.5cm), large microplastics (1mm to 5mm), small microplastics (1µm to 1mm) and nanoplastics (1nm to 1µm).

3.3 Analyses of the amount of plastics present in seabirds with different foraging distributions

Based on the data from the different foraging distributions, pelagic and coastal, it was possible to find differences in the occurrence of potential plastics (Table 12). In terms of plastics presence there were no significant differences between the species that forage in both areas. Species that forage in pelagic areas showed significantly more fragments ($\beta = 0.658 \pm 0.245$; $Z = 2.688$; $p = 0.007$), mesoplastics ($\beta = 1.397 \pm 0.567$; $Z = 2.464$; $p = 0.014$) and pink plastics ($\beta = 1.980 \pm 0.626$; $Z = 3.165$; $p = 0.002$) presence than species that forage in coastal areas (the reference category). Samples from species that forage in pelagic areas presented significantly lower frequency of fibers ($\beta = -1.247 \pm 0.237$; $Z = 5.268$; $p < 0.001$), large microplastics ($\beta = -0.661 \pm 0.261$; $Z = 2.533$; $p = 0.011$) and orange plastics ($\beta = -2.996 \pm 1.036$; $Z = 2.891$; $p = 0.004$) than coastal foraging species.

When assessing differences in the number of plastics among distributions most of the categories fitted better with a Poisson distribution, but "total number", "number of blue plastics", "number of small microplastics", "number of large microplastics", "number of fragments" and "number of fibers" fitted better with zero inflated models with a negative binomial distribution fitting. No significant differences were found between both foraging habitats for total number of plastics neither to plastic types. Pelagic foraging seabirds presented significantly higher number of mesoplastics ($\beta = 1.687 \pm 0.624$; $Z = 2.705$; $p = 0.007$) and pink plastics ($\beta = -2.016 \pm 0.611$; $Z = 3.299$; $p = 0.001$) than the reference species. Species that forage in pelagic areas presented a lower number of purple ($\beta = -0.931 \pm 0.453$; $Z = 2.055$; $p = 0.040$) and orange ($\beta = -2.938 \pm 1.029$; $Z = 2.855$; $p = 0.004$) plastics than in coastal foraging species (Table 13 and Fig. 23).

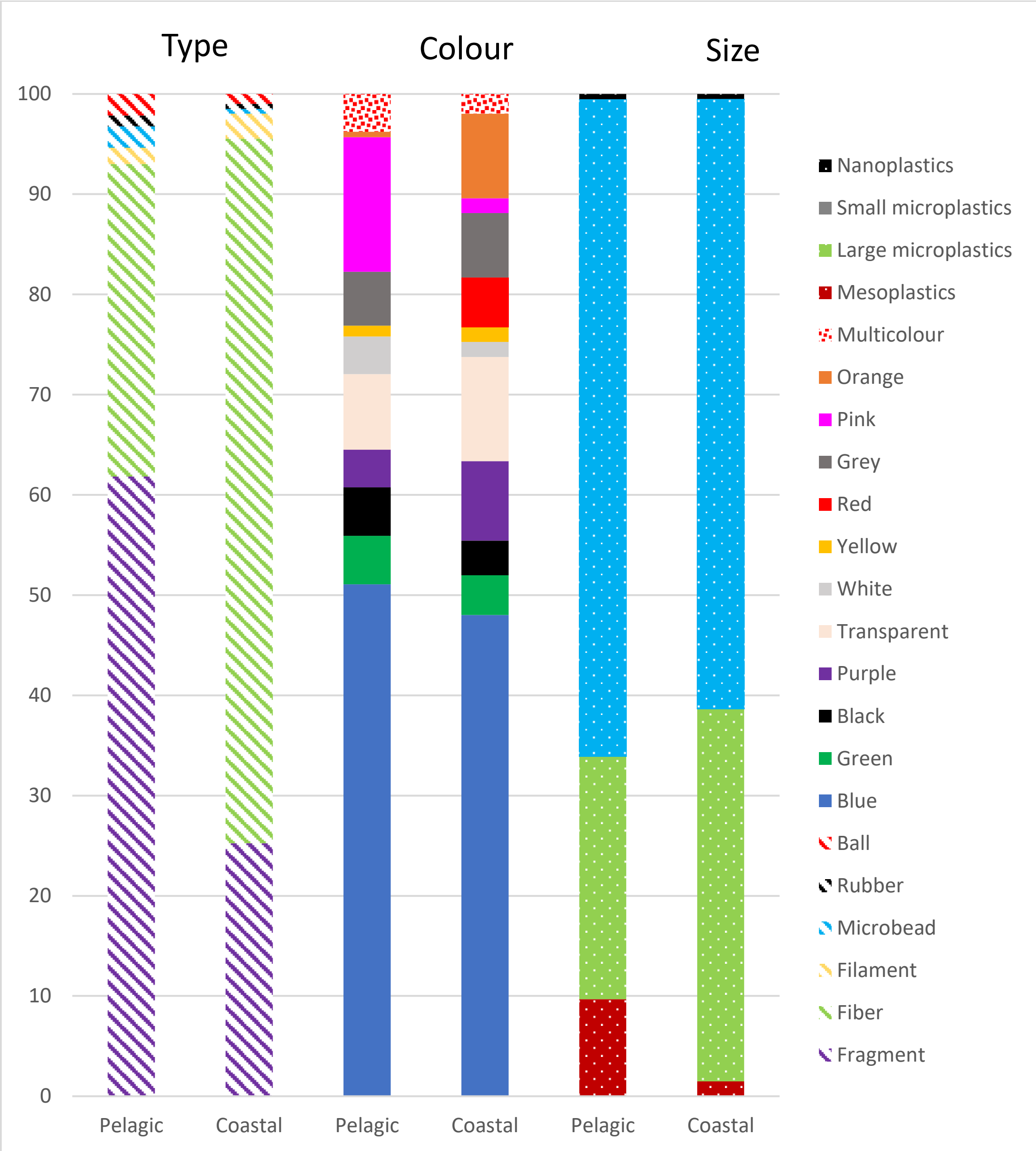

Figure 23. Numeric frequency bar chart comparing species that forage in coastal areas (N = 129; number of plastics = 202): *Ichthyaehus audouinii*, *Calonectris borealis*, *Sula leucogaster* and *Phaethon aethereus*, and pelagic areas (N = 215; number of plastics = 186): *Calonectris edwardsii*, *Bulweria bulwerii and Puffinus lherminierii boydi*. Sizes are divided into mesoplastics (5mm to 2.5cm), large microplastics (1mm to 5mm), small microplastics (1µm to 1mm) and nanoplastics (1nm to 1µm).

3.4 Analyses of the amount of plastics present in seabirds in relation to their interaction with fisheries

Based on the data that compares species that interact with fisheries with species that do not interact, it was possible to find differences in the occurrence of potential plastics (Table 14). In terms of presence, the species that do not interact with fisheries had significantly ($\beta = -0.585 \pm 0.234$; $Z = 2.497$; $p = 0.013$) less plastics than the species that interact with fisheries (the reference category). Species that do not interact with fisheries also presented significantly less fibers ($\beta = -0.807 \pm 0.233$; $Z = 3.458$; $p = 0.001$), mesoplastics ($\beta = -2.494 \pm 0.635$; $Z = 3.929$; $p < 0.001$), large microplastics ($\beta = -0.982 \pm 0.263$; $Z = 3.740$ $p < 0.001$), blue ($\beta = -0.839 \pm 0.233$; $Z = 3.598$ $p < 0.001$) and transparent plastics ($\beta = -0.765 \pm 0.385$; $Z = 1.987$; $p = 0.047$) than the reference species.

The best fitted model for most of the categories was the Poisson distribution but "total number" and "number of fragments" data had a better fit with zero inflated models with a negative binomial distribution fitting. The categories, "number of small microplastics", "number of large microplastics" and "number of fibers" had a better fit with a zero negative distribution. In relation to the total numbers of plastics, there were no significant differences between both group of species. Species that do not interact with fisheries showed significantly less quantity of fragments ($\beta = -0.951 \pm 0.248$; $Z = 3.834$ $p < 0.001$), mesoplastics ($\beta = -2.328 \pm 0.624$; $Z = 3.732$; $p < 0.001$) and transparent plastics ($\beta = -0.823 \pm 0.342$; $Z = 2.411$; $p = 0.016$) than species that interact with fisheries. (Table 15 and Fig. 24)

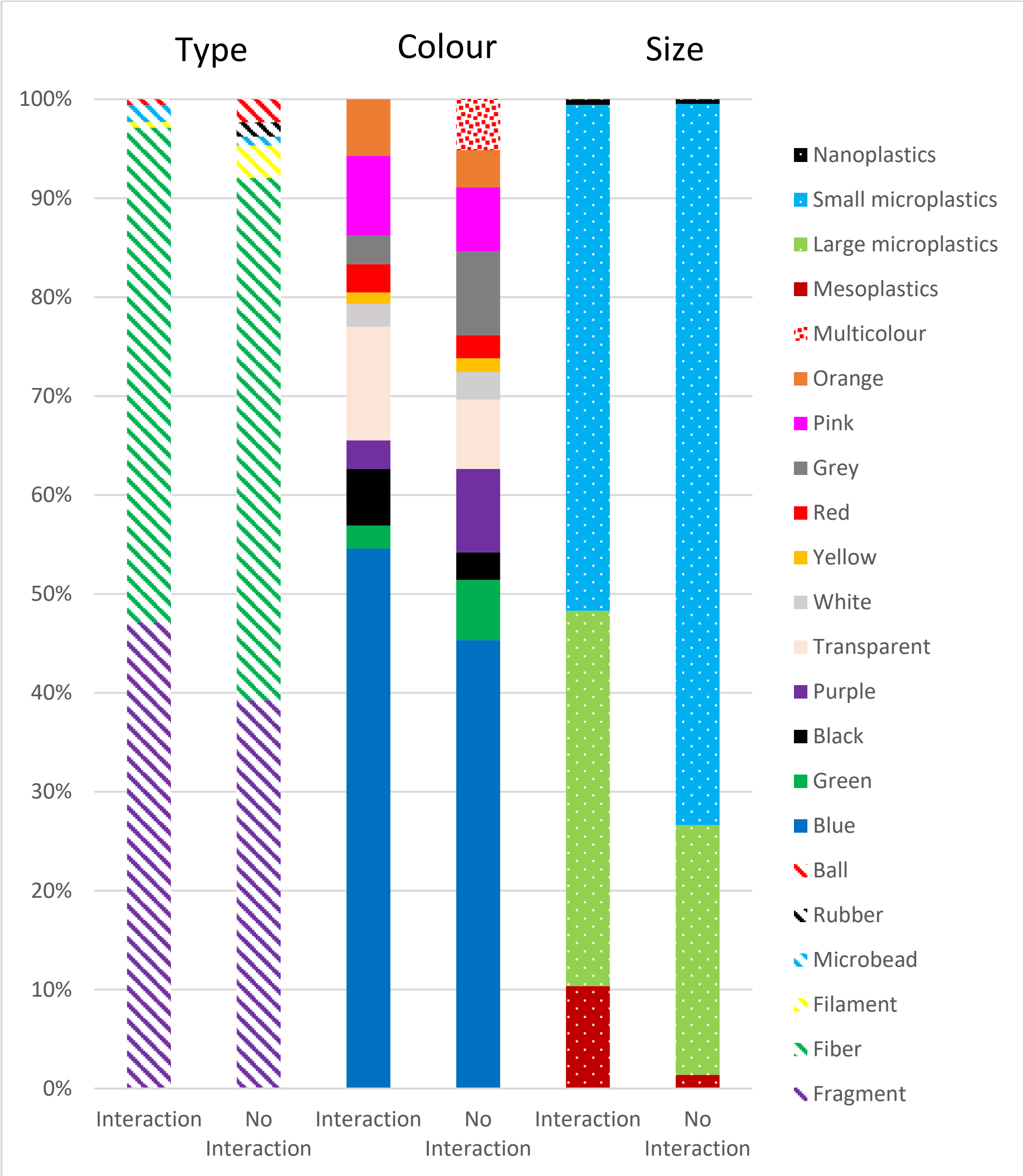

Figure 24. Numeric frequency bar chart comparing species that interact with fisheries (N = 127; number of plastics = 174): *Ichthyaehus audouinii*, *Calonectris borealis*, *Calonectris edwardsii*, with species that do not interact with fisheries (N = 217; number of plastics = 214): *Phaethon aethereus*, *Sula leucogaster*, *Bulweria bulwerii* and *Puffinus lherminierii boydi*. Sizes are divided into mesoplastics (5mm to 2.5cm), large microplastics (1mm to 5mm), small microplastics (1µm to 1mm) and nanoplastics (1nm to 1µm).

3.5 Analyses of the amount of plastics present in seabirds with different sizes

There were no significant differences in the occurrence of potential plastics in relation to the size of seabird species (Table 16). In terms of plastics presence, the small sized species presented significantly less plastics ($\beta = -1.043 \pm 0.343$; $Z = 3.043$; $p = 0.002$) than large sized species (the reference category). Small sized seabirds presented significantly lower occurrence of fibers ($\beta = -2.055 \pm 0.371$; $Z = 5.541$; $p < 0.001$), large microplastics ($\beta = -1.595 \pm 0.430$; $Z = 3.706$; $p < 0.001$), small microplastics ($\beta = -0.999 \pm 0.333$; $Z = 3.000$; $p = 0.003$), blue ($\beta = -1.202 \pm 0.353$; $Z = 3.406$; $p = 0.001$), purple ($\beta = -1.658 \pm 0.727$; $Z = 2.280$; $p = 0.023$) and grey plastics ($\beta = -2.374 \pm 0.675$; $Z = 3.518$; $p < 0.001$) than large sized species. Medium sized species presented less significantly lower frequency of filaments ($\beta = -2.531 \pm 1.130$; $Z = 2.240$; $p = 0.025$) and grey plastics ($\beta = -1.568 \pm 0.496$; $Z = 3.162$; $p = 0.002$) than large sized species.

When assessing differences in the number of plastics among seabirds sizes most of the categories fitted better with a Poisson distribution, whilst “total number”, “number of blue plastics”, “number of large microplastics” and “number of fibers” data presented a better fit with zero inflated models with a negative binomial distribution fitting. The categories “number of fragments” and “number of small microplastics” fitted better with a zero negative distribution. Small sized species presented a significantly smaller number of plastics ($\beta = -1.021 \pm 0.236$; $Z = 4.335$; $p < 0.001$) than the reference species. Small sized species presented a significantly lower number of filaments ($\beta = -1.787 \pm 0.837$; $Z = 2.136$; $p = 0.033$), small microplastics ($\beta = -0.868 \pm 0.328$; $Z = 2.647$; $p = 0.008$), purple ($\beta = -1.564 \pm 0.707$; $Z = 2.212$; $p = 0.027$) and grey plastics ($\beta = -1.659 \pm 0.539$; $Z = 3.076$; $p = 0.002$) than the large sized species. Medium sized species presented a higher number of fragments ($\beta = 0.984 \pm 0.413$; $Z = 2.384$; $p = 0.017$) than the reference species, but also presented a significantly lower quantity of filaments ($\beta = -2.702 \pm 1.095$; $Z = 2.466$; $p = 0.014$) and grey plastics ($\beta = -1.544 \pm 0.484$; $Z = 3.194$; $p = 0.001$) than large sized (Table 17 and Fig. 25).

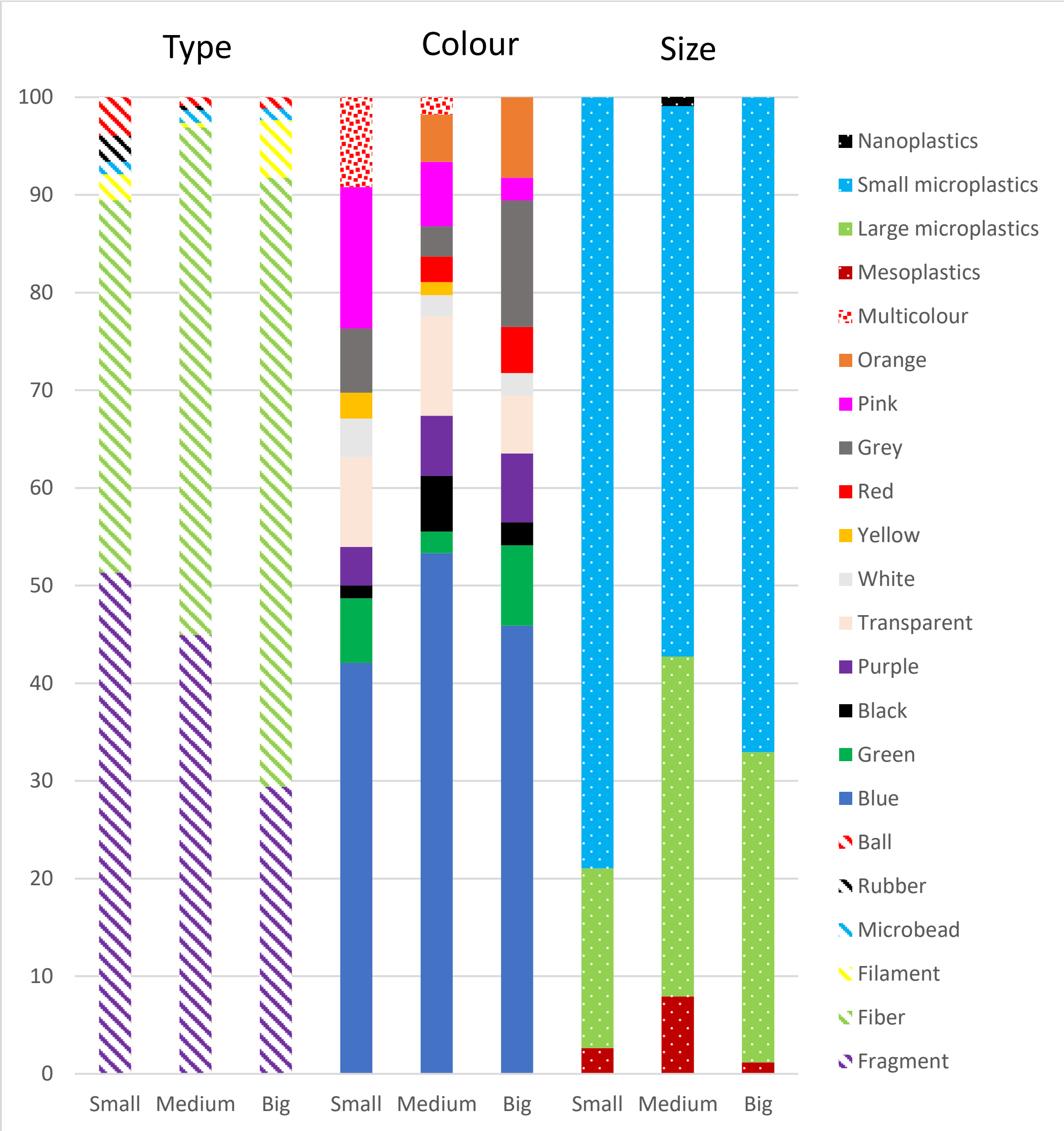

Figure 25. Numeric frequency bar chart comparing species with different size, small birds (N = 129; number of plastics = 76): *Bulweria bulwerii* and *Puffinus lherminierii boydi*, medium birds (N = 161; number of plastics = 227): *Ichthyaehus audouinii*, *Calonectris borealis*, *Calonectris edwardsii* and *Phaethon aethereus*, big birds (N = 54; number of plastics = 85): *Sula leucogaster*. Plastic sizes are divided into mesoplastics (5mm to 2.5cm), large microplastics (1mm to 5mm), small microplastics (1µm to 1mm) and nanoplastics (1nm to 1µm).

CHAPTER IV – Discussion

4.1 The effect of seabird taxonomy and foraging ecology in plastic ingestion

In this study we assessed the frequency of occurrence and numeric frequency of plastics on faeces samples of seabird species from Portugal and Cabo Verde using faeces as a proxy of ingestion. Among a total of 344 faecal samples, 57.0% of them contained plastics, and a total of 388 possible plastics were found and analyzed. Results showed the presence of potential plastics in the seven studied species, although with different mean frequency of occurrence among them (*Ichthyaehus audouinii,* IA = 62.5%; *Calonectris borealis,* CB = 41.9%; *Calonectris edwardii, CE* = 86.5%; *Phaethon aethereus, PA* = 64.7%; *Sula leucogaster, SL* = 68.5%; *Bulweria bulwerii,* BB = 35.9%; *Puffinus iherminieri boydi,* PB =44.4%) across 4 different taxonomic orders.

Regarding taxonomy, several studies reported Procellariiformes to be the seabird order with comparably higher frequency of occurrence of plastics in their diet (Colabuono *et al.* 2009; Rodríguez *et al*. 2012; Codina-garcía *et al*. 2013; Hyrenbach & Hester, 2014; Roman *et al.* 2016) . Interestingly, the results showed that not all Procelllariiforms exhibited higher number of plastics comparing with the other species and orders. As expected, CE was the species with more plastics in their diet though, contrary to the expectations PB, also a Procellariiform, was the species that presented lower quantity, this result does not concur with studies that identify *Puffinus* sp. as a species vulnerable to plastic ingestion (Vlietstra & Parga, 2002).

Gulls have the ability to regurgitate and Codina-garcía *et al.* 2013 associated this ability with the low quantity of plastics found in this species. This relation was partly visible in this study because IA, which also regurgitates, exhibited less plastics than species that do not regurgitate (e.g. CE). In terms of plastic size, IA presented more plastics with higher dimension than the remain seabird species. Procellariformes have a unique gizzard morphology (Furness, 1985) making it impossible to regurgitate hard remains from their prey, which justifies the high presence of plastics in CE faeces. However, PB, which is also a Procellariiformes, had a lower amount of plastics than the species that regurgitate. This means that, apart from the ability to regurgitate, the species-specific foraging ecology and distribution should be important in explaining the presence and quantity of plastics in faecal samples (Wilcox *et al.* 2015; Roman *et al.* 2019).

Gilbert *et al.* 2016 compared the number of plastics ingested between coastal (birds that forage along the shoreline or intertidal coastal environments) and pelagic (birds that forage in open ocean) species, comparing Laridae and Sulidae with Procellariiformes. The results obtained in this study are in line with former studies (Roman *et al.* 2016) and show that pelagic foraging seabirds ingest a higher quantity of plastics than coastal foraging seabirds. Results showed that there were no significant differences between both foraging habitat plastic presence and quantity in the samples. It was only possible to verify that coastal foraging species had a higher presence of fibers in their samples and pelagic foraging species possessed a significantly higher amount of fragments. However, when analysing the species individually it is possible to verify that CE had the higher presence of plastics and CB was one of the species presenting less plastics presence, taking into account that this species forage in both areas and cover a vast territory (Paiva *et al.* 2015; Gilbert *et al.* 2016), it allows no conclusion about which foraging distribution provides more plastic because they may ingest plastic from both. Concerning only the species that forage in one area, results showed disagreement with the previously mentioned studies. The coastal species (IA, PA and SL) showed higher plastic prevalence than seabirds foraging just in pelagic areas (BB and PB). Coastal foraging species also presented considerably more fibers and orange plastics than pelagic species which presented more fragments and pink plastics.

One of the expected results of this study was that larger seabirds would exhibit comparably higher amount of plastics, and those plastics would have larger dimensions than the plastics present in the smaller species (Hyrenbach & Hester, 2014). In this study there was no correlation between bird mass and the presence of plastic, and this lack of correlation has been regularly reported in other studies (Codina-garcía *et al.* 2013; Gilbert *et al.* 2016). On the other hand, small sized species presented significantly less small microplastics, than the medium and large sized species, which was also not expected. Also, the expected positive correlation between sample weight and plastics' number was also not significant. However, the possibility of this correlation to be significant with a larger sample size may not be discarded (i.e. p-value = 0.07).

It has been reported that fishery activities are the responsible of a high amount of oceanic plastic pollution (Li *et al.* 2016) and some seabird species, including IA, CB and CE, are known to interact with fisheries (Paiva *et al*. 2015; Matos *et al.* 2018; Pereira *et al.* 2021). Thus, an expected result was that these species should have a higher presence of plastics namely fibers and fragments, of the colours blue, transparent and green, which are derived from the degradation of fishing ropes (Possatto *et al*. 2011). Indeed, the former species exhibited more plastics of colours blue and transparent (Vries *et al.* 2020). Looking at each species individually it was possible to notice that CE, one of the species that interacts with fisheries, presented the higher presence and quantity of plastics, and significantly more blue plastics than the other species. Furthermore, IA was the species that presented higher percentage of transparent plastics. Both these results may be due to the interaction with fisheries. The high quantity of blue plastics found in the samples can be also connected to the known fish ingestion of blue-coloured plastics, mistaking it with their preys (Ory *et al.* 2017; Schirinzi *et al.* 2020).

4.2 Prevalence of plastic contamination on seabirds from temperate and tropical regions

Cabo Verde has a complex oceanographic context as it is affected by the North Equatorial and Mauritanian Currents (Cardoso & Caldeira, 2021). The North Equatorial Current comes from the Northwestern African coast, and although the countries of this region have a low coastal population density, their poor waste management policies and poor infrastructures make them an important supplier of plastic into the ocean. Jambeck *et al.* 2015 and Worm *et al.* 2017 showed that Morocco produces as much plastic marine debris as all the coastal European union countries combined, thus affecting the marine environment of the Cabo Verde archipelago. Results from this study showed that this contamination in Cabo Verde presumably led to a higher ingestion of plastics by tropical seabird species which breed in this region. Another interesting result of this study was that faecal samples from Cabo Verde seabirds had a significantly higher prevalence of small microplastics when compared to seabirds breeding in Portugal, and this high presence of small sized microplastics in the archipelago was already noticed by Cardoso & Caldeira, 2021.

Portugal presents also a high quantity of plastic in the ocean because it is a touristic country, especially the Algarve region where Ria Formosa is integrated. It is located in one of the main shipping corridors, making the connection between the Mediterranean, the Northern Europe and the Western Atlantic (Sá *et al.* 2016). In 2021, the country had a fishing fleet of over 7500 vessels (Instituto Nacional de Estatística, 2021). The former three factors combined likely contributed to the prevalence of plastics in the samples of seabirds inhabiting this region.

Overall, the patterns of plastic pollution depicted by different seabird species from the temperate and tropical regions should also be related with different debris management, which is generally better in the European continent.

4.3 Seabirds as sentinels of coastal and oceanic plastic pollution

The use of plastic grew exponentially in recent decades which likely translated in increasingly amounts of plastics reaching and concentrating on the marine environment. This accumulation is ubiquitous, but it is an even higher threat to islands as they are exposed to plastics from other regions. The plastics' impacts are multiples and can affect socio-economic sectors such as tourism, fisheries and shipping industries, from which islands are usually dependent on (Rodríguez *et al.* 2020). Moreover, they can cause harm to organisms and consequently to ecosystems. Hence, knowing the consequences of plastics in the marine environment is key to latter delineate actions that might mitigate their impact. Seabirds have been used as indicators of climatic alterations and perturbations caused by human on marine ecosystems (Paiva, 2022). This study contributes to support the pertinence and usefulness of using coastal and pelagic seabirds also as indicators of plastic pollution in the marine realm (Provencher *et al*. 2019; Ramos & Pereira, 2022).

Main goal of this project was to evaluate the role of taxonomy, feeding ecology and distribution on the plastic ingestion of seabird species. All studied seabird species suffered from plastic pollution, with some species showing a higher plastic prevalence than others. This variability was noticeable in the plastic presence/absence, plastic quantity, plastic type, plastic size and plastic colour. Such variability was likely influenced by bird size, taxonomic group, habitat, foraging range and interaction with fisheries. The relationship between these variables and the plastic ingestion is the key to better understand and predict which species are at higher risk/ exposure of ingesting plastic particles (Roman *et al.* 2016). In Portugal both CB and IA had similar patterns of plastic ingestion, making them equally suitable as sentinels of plastic pollution. In Cabo Verde all the species contained plastics, although CE was the species showing a higher prevalence, which turns this species of high value as a sentinel of plastic pollution in the region. Differences between species of the same order show that the taxonomy itself is not a determinant factor defining patterns of plastic ingestion by seabirds, the species' foraging behaviour and distribution are likely more relevant drivers of different plastic ingestion rates in taxonomy-related taxa.

This project had some limitations. A Fourier-transform infrared spectroscopy (FTIR) would avoid misidentification of natural items for plastics (Nicastro *et al.* 2018), but was impossible to perform in this study samples due to technical issues with the equipment. This is of extreme importance for microplastics studies due to the difficulty to distinguish plastic material from other substances, and it would also provide us the capacity to better understand the origin and path of the plastics. The aerial contamination in the laboratory is impossible to deny, and even with all the control measures used in this project some contamination was found. This contamination was excluded when observing through the stereomicroscope in order to discard all the contamination, but it is possible that some plastics were also excluded by mistake, so the results can be underestimated. In this project faecal samples were used to analyse seabird plastic consumption, but other methods may also be used such as necropsy of birds carcasses, study of the bolus and regurgitated material (Provencher *et al.* 2017). All the methods have advantages and disadvantages. In the necropsy it is possible to analyse plastics all over the body, and to correlate the results with the sex, age and possible death causes of the bird, but has the disadvantage of non-control of the samples and possible sampling bias, because the collected individuals could have died due to plastic interactions complications (Codina-garcía *et al.* 2013). Sampling of bolus is advantageous as it can be made systematically but it is very invasive to the organism (Provencher *et al.* 2017). The plastic particles can be retained in the gizzard until they get a size physically capable to pass through the intestines and then be excreted (Provencher *et al*. 2018). The faecal sampling method is advantageous as it is less prejudicial to the specimens and can be considered a random sampling method with no selection bias regarding the content of plastics. The most complete results would come from the conjugation of the three methods. Another limitation of this project was that the number of samples collected from two species (IA and PA) was low (less than 40 samples) and, with a higher number of samples, the results would be more accurate and complete.

The results supported the theory that the plastic contamination is a ubiquitous and alarming issue. It is essential to collect data on plastic availability. This data can be provided by trawls, visual counts, beach surveys and wildlife analyses (Pedrotti *et al*. 2022). Organisms may be used as indicators of plastic presence and abundance, and seabirds are very good sentinels as they are top predators, forage in wide range of areas and provide worldwide comparable results, but it is also urgent to assess the effects on their health, particularly in the case of the threatened species. A long-term monitoring program should be implemented with standardized methods (GESAMP 2015; Bessa *et al.* 2019), enabling to better inform and answer the questions about the impact of plastics and to compare results at different temporal and spatial scales. An understanding of these patterns can help define conservation priorities and reach a better conservation status of the species. Awareness campaigns and stricter laws about plastic usage and waste management should be a priority. These measures need to be well studied and applied because the high quantity of plastics found in wildlife correspond to a direct relation between the animals with the human actions such as fisheries. The society can also implement some actions such as less plastic usage, plastic recycling and also beach clean-ups helping to reach the main goal, a less contaminated world with healthier wildlife.

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Annexes

Table 6. Frequency of occurrence of plastic types, size (mesoplastics (5mm to 2.5cm), large microplastics (1mm to 5mm), small microplastics (1µm to 1mm) and nanoplastics (1nm to 1µm)) and colours of plastics detected in the faeces of seven seabird species. N is the number of birds of each species.

				Type			
Species	**N**	**Fragment %**	**Fiber %**	**Filament %**	**Microbead %**	**Rubber %**	**Ball %**
Ichthyaetus audouinii	32	25.0	95.0	—	—	—	—
Calonectris borealis	43	5.6	100	—	—	—	—
Calonectris edwardsii	52	75.6	46.7	2.2	6.7	—	2.2
Phaethon aethereus	34	54.6	63.6	—	—	4.6	4.6
Sula leucogaster	54	51.4	83.8	10.8	2.7	—	2.7
Bulweria bulwerii	39	71.4	50.0	7.1	—	—	7.1
Puffinus lherminieri boydi	90	52.5	35.0	2.5	2.5	5.0	5.0
				Size			
Species	**N**	**Mesoplastic %**	**Large Microplastic %**	**Small Microplastic %**	**Nanoplastic %**		
Ichthyaetus audouinii	32	—	80.0	45.0	—		
Calonectris borealis	43	11.1	38.9	66.7	—		

Calonectris edwardsii	52	31.1	44.4	71.1	2.2
Phaethon aethereus	34	—	36.4	90.9	4.6
Sula leucogaster	54	2.7	46.0	88.2	—
Bulweria bulwerii	39	7.1	7.1	100	—
Puffinus lherminieri boydi	90	2.5	25.0	82.5	—

		Colour											
Species	**N**	**Blue %**	**Green %**	**Black %**	**Purple %**	**Transparent %**	**White %**	**Yellow %**	**Red %**	**Grey %**	**Pink %**	**Orange %**	**Multicolour %**
Ichthyaetus audouinii	32	60.0	—	5.0	—	45.0	—	5.0	15.0	—	—	45.0	—
Calonectris borealis	43	66.7	—	5.6	5.6	5.6	—	5.6	11.1	—	—	—	—
Calonectris edwardsii	52	77.8	6.7	6.7	8.9	13.3	6.7	—	—	11.1	24.4	2.2	—
Phaethon aethereus	34	72.7	4.6	13.6	31.8	13.6	4.6	4.6	4.6	9.1	4.6	4.6	18.2
Sula leucogaster	54	64.9	18.9	2.7	16.2	10.8	2.7	—	10.8	29.7	5.4	16.2	—
Bulweria bulwerii	39	64.3	—	—	7.1	28.6	7.1	7.1	—	—	14.3	—	—
Puffinus lherminieri boydi	90	40.0	12.5	2.5	5.0	7.5	5.0	2.5	—	7.5	22.5	—	15.0

Table 7. Generalized linear model testing the effect of seabird species and plasctic presence, type, colour and size (mesoplastics (MP; 5mm to 2.5cm), large microplastics (LMP; 1mm to 5mm), small microplastics (SMP; 1µm to 1mm) and nanoplastics (NP; 1nm to 1µm)). The table present the best fit model of each category. Models with significant results are shown on the table 4.

Species	Count model	Filament	Microbead	Rubber	Ball	Purple	Grey	Pink	Multicolour
Calonectris borealis	β ± SE	-6.953e-07 ± 4.205e+03	7.537e-13 ± 6.933e+03	2.850e-13 ±1.143e+04	2.473e-08 ± 4.205e+03	14.900 ± 1153.050	-5.516e-13 ±2.551e+03	-6.617e-08 ±2.551e+03	-2.157e+01 ± 5.168e+03
	Z	0.000	0.000	0.000	0.000	0.013	0.000	0.000	0.004
	P	1.000	1.000	1.000	1.000	0.990	1.000	1.000	0.997
Calonectris edwardsii	β ± SE	1.663e+01 ± 3.134e+03	1.877e+01 ± 5.168e+03	2.703e-13 ±1.083e+04	1.663e+01 ± 3.134e+03	16.080 ± 1153.050	1.733e+01 ±1.901e+03	1.825e+01 ±1.901e+03	2.766e-13 ± 6.933e+03
	Z	0.005	0.004	0.000	0.005	0.014	0.009	0.010	0.000
	P	0.996	0.997	1.000	0.996	0.989	0.993	0.992	1.000
Phaethon aethereus	β ± SE	-6.954e-07 ±4.367e+03	6.012e-13 ±7.200e+03	1.907e+01 ±8.520e+03	1.707e+01 ±3.134e+03	17.220 ± 1153.050	1.723e+01 ±1.901e+03	1.607e+01 ±1.901e+03	1.064e-13 ±6.568e+03
	Z	0.000	0.000	0.002	0.005	0.015	0.009	0.008	0.000
	P	1.000	1.000	0.998	0.996	0.988	0.993	0.993	1.000
Sula leucogaster	β ± SE	1.804e+01 ± 3.134e+03	1.760e+01 ± 5.168e+03	1.763e-13 ±1.075e+04	1.660e+01 ± 3.134e+03	16.490 ± 1153.050	1.820e+01 ±1.901e+03	1.631e+01 ±1.901e+03	1.955e+01 ± 5.168e+03
	Z	0.006	0.003	0.000	0.005	0.014	0.010	0.009	0.004
	P	0.995	0.997	1.000	0.996	0.989	0.992	0.993	0.997
Bulweria bulwerii	β ± SE	1.693e+01 ±3.134e+03	-1.973e-10 ±6.972e+03	-1.351e-10 ±1.150e+04	1.693e+01 ±3.134e+03	14.930 ± 1153.050	8.794e-11 ±2.565e+03	1.665e+01 ±1.901e+03	3.624e-10 ±6.972e+03
	Z	0.005	0.000	0.000	0.005	0.013	0.000	0.009	0.000
	P	0.996	1.000	1.000	0.996	0.990	1.000	0.993	1.000
Puffinus lherminieri boydi	β ± SE	1.608e+01 ±3.134e+03	1.708e+01 ±5.168e+03	1.878e+01 ±8.520e+03	1.678e+01 ±3.134e+03	14.780 ± 1153.050	1.620e+01 ±1.901e+03	1.737e+01 ±1.901e+03	1.893e+01 ±5.168e+03
	Z	0.005	0.003	0.002	0.005	0.013	0.009	0.009	0.004
	P	0.996	0.997	0.998	0.996	0.990	0.993	0.993	0.997

Table 8. Numeric frequency of all the species with the plastic type, size (mesoplastics (5mm to 2.5cm), large microplastics (1mm to 5mm), small microplastics (1µm to 1mm) and nanoplastics (1nm to 1µm)) and colour. N is the total number of plastics of each species.

				Type			
Species	**N**	**Fragment %**	**Fiber %**	**Filament %**	**Microbead %**	**Rubber %**	**Ball %**
Ichthyaetus audouinii	39	12.8	87.2	—	—	—	—
Calonectris borealis	25	4.0	96.0	—	—	—	—
Calonectris edwardsii	111	68.1	26.1	0.9	2.7	0.9	0.9
Phaethon aethereus	53	37.7	58.5	—	—	1.9	1.9
Sula leucogaster	86	29.1	61.6	5.8	1.2	1.2	1.2
Bulweria bulwerii	22	54.6	31.8	4.6	—	4.6	4.6
Puffinus lherminieri boydi	55	49.1	40.0	1.8	1.8	3.6	3.6

			Size		
Species	**N**	**Mesoplastic %**	**Large Microplastic %**	**Small Microplastic %**	**Nanoplastic %**
Ichthyaetus audouinii	36	—	66.7	33.3	—
Calonectris borealis	25	8.0	32.0	60.0	—
Calonectris edwardsii	110	14.6	28.2	56.4	0.9
Phaethon aethereus	53	—	24.5	73.6	1.9

Sula leucogaster	84	1.2	32.1	66.7	—
Bulweria bulwerii	21	4.8	4.8	90.5	—
Puffinus lherminieri boydi	55	1.8	23.6	74.6	—

		Colour											
Species	**N**	**Blue %**	**Green %**	**Black %**	**Purple %**	**Transparent %**	**White %**	**Yellow %**	**Red %**	**Grey %**	**Pink %**	**Orange %**	**Multicolour %**
Ichthyaetus audouinii	39	35.9	—	2.6	—	28.2	—	2.6	7.7	—	—	23.1	—
Calonectris borealis	25	72.0	—	4.0	4.0	8.0	—	4.0	8.0	—	—	—	—
Calonectris edwardsii	110	52.3	3.6	7.3	3.6	6.4	3.6	—	—	4.5	12.7	0.9	—
Phaethon aethereus	53	49.1	1.9	5.7	17.0	5.7	1.9	1.9	1.9	3.8	1.9	1.9	7.6
Sula leucogaster	85	45.9	8.2	2.4	7.1	5.9	2.4	—	4.7	12.9	2.4	8.2	—
Bulweria bulwerii	21	57.1	—	—	4.8	19.1	4.8	4.8	—	—	9.5	—	—
Puffinus lherminieri boydi	55	36.4	9.1	1.8	3.6	5.5	3.6	1.8	—	9.1	16.4	—	12.7

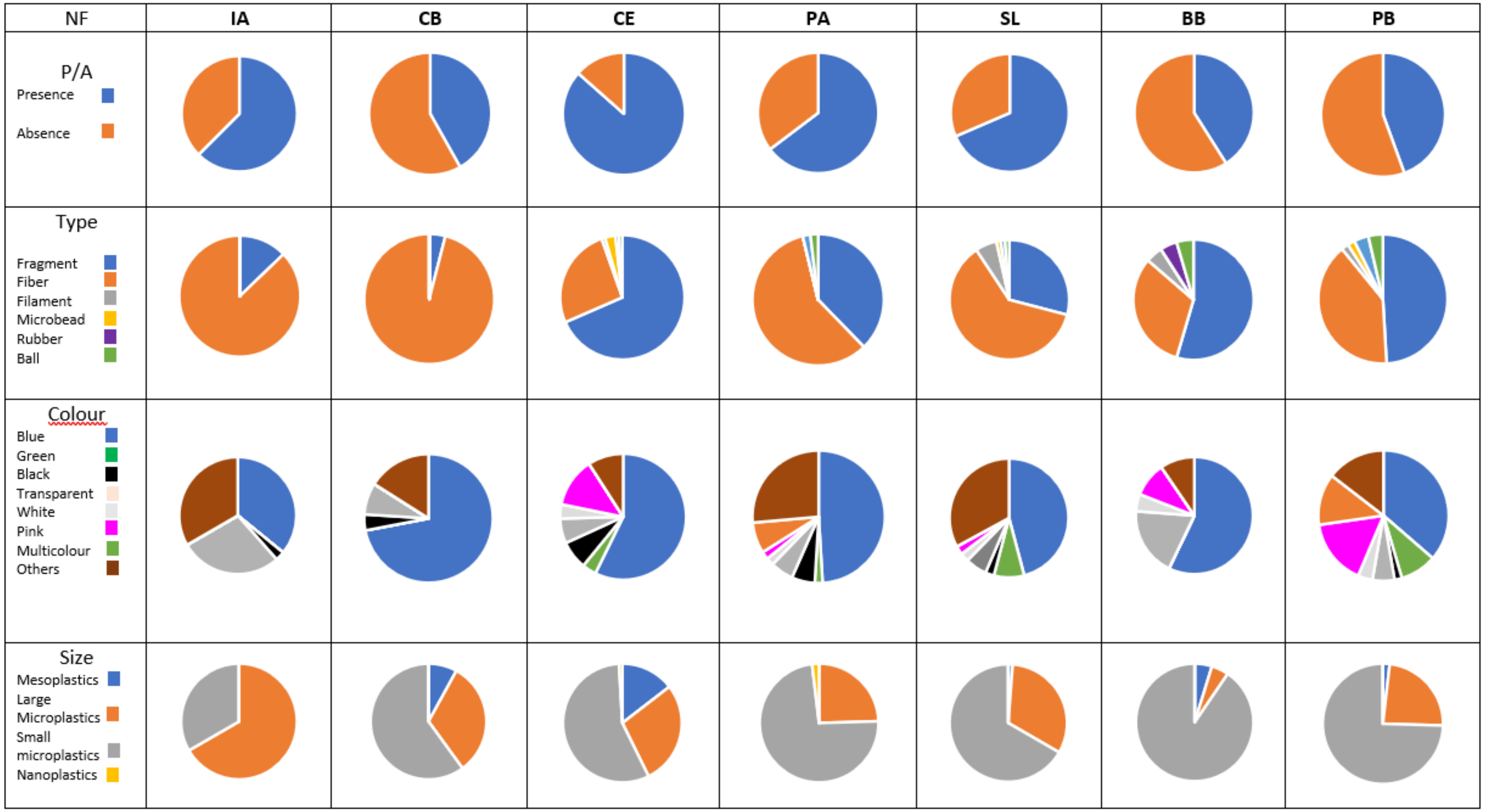

Figure 25. Numeric frequency pie charts type, colour and size (mesoplastics (5mm to 2.5cm), large microplastics (1mm to 5mm), small microplastics (1µm to 1mm) and nanoplastics (1nm to 1µm)) in *Ichthyaetus audouinii* (IA; N = 32, number of plastics = 39), *Calonectris borealis* (CB; N = 43, number of plastics = 25), *Calonectris edwardsii* (CE; N = 52, number of plastics = 111), *Phaethon aethereus* (PA; N = 34, number of plastics = 53), *Sula leucogaster* (SL; N = 54, number of plastics = 86), *Bulweria bulwerii* (BB; N = 39, number of plastics= 22), *Puffinus lherminieri boydi* (PB; N = 90, number of plastics = 55).

Table 9. Generalized linear model testing the interaction between species and plasctics' number, type, colour size (mesoplastics (MP; 5mm to 2.5cm), large microplastics (LMP; 1mm to 5mm), small microplastics (SMP; 1µm to 1mm) and nanoplastics (NP; 1nm to 1µm)) of numeric frequency data applying Poisson distribution, zero inflated model and zero inflated with negative binomial distribution model. The table presents the best fit model of each category. The table present the best fit model of each category. Models with significant results are shown on the table 5.

Species	Count model	Fiber	Filament	Microbead	Rubber	Ball	Purple	Grey	Pink	Multicolour	MP
Calonectris borealis	β ± SE	-0.695 ± 0.385	-9.354e-08 ±5.982e+03	-5.575e-13 ±5.982e+03	-1.054e-12 ±9.863e+03	2.052e-09 ±3.628e+03	15.540 ±1666.410	1.058e-12 ±3.628e+03	3.437e-08 ±2.201e+03	-1.059e-13 ±5.982e+03	1.723e+01 ±2.747e+03
	Z	1.808	0.000	0.000	0.000	0.000	0.009	0.000	0.000	0.000	0.006
	P	0.071	1.000	1.000	1.000	1.000	0.993	1.000	1.000	1.000	0.995
Calonectris edwardsii	β ± SE	-0.679 ± 0.463	1.735e+01 ±4.530e+03	1.845e+01 ±4.530e+03	-7.013e-13 ±9.492e+03	1.635e+01 ±2.747e+03	16.740 ±1666.410	1.796e+01 ±2.747e+03	1.799e+01 ±1.666e+03	-2.558e-13 ±5.757e+03	1.912e+01 ±2.747e+03
	Z	1.467	0.004	0.004	0.000	0.006	0.010	0.007	0.011	0.000	0.007
	P	0.142	0.997	0.997	1.000	0.995	0.992	0.995	0.991	1.000	0.994
Phaethon aethereus	β ± SE	0.391 ± 0.394	-9.346e-08 ±6.311e+03	2.268e-13 ±6.311e+03	1.878e+01 ±7.468e+03	1.678e+01 ±2.747e+03	17.970 ±1666.410	1.747e+01 ±2.747e+03	1.578e+01 ±1.666e+03	1.916e+01 ±4.530e+03	-4.691e-07 ±3.828e+03
	Z	0.992	0.000	0.000	0.003	0.006	0.011	0.006	0.009	0.004	0.000
	P	0.321	1.000	1.000	0.998	0.995	0.991	0.995	0.992	0.997	1.000
Sula leucogaster	β ± SE	-0.098 ± 0.363	1.892e+01 ±4.530e+03	1.731e+01 ±4.530e+03	-2.493e-13 ±9.425e+03	1.631e+01 ±2.747e+03	17.110 ±1666.410	1.871e+01 ±2.747e+03	1.601e+01 ±1.666e+03	-6.230e-13 ± 5.716e+03	1.631e+01 ±2.747e+03
	Z	0.270	0.004	0.004	0.000	0.006	0.010	0.007	0.010	0.000	0.006
	P	0.787	0.997	0.997	1.000	0.995	0.992	0.995	0.992	1.000	0.995
Bulweria bulwerii	β ± SE	-0.047 ± 0.686	1.764e+01 ±4.530e+03	-1.829e-11 ±6.112e+03	-3.782e-10 ±1.008e+04	1.664e+01 ±2.747e+03	15.640 ±1666.410	1.933e-10 ±3.707e+03	1.633e+01 ±1.666e+03	-6.488e-11 ±6.112e+03	1.664e+01 ±2.747e+03
	Z	0.069	0.004	0.000	0.000	0.006	0.009	0.000	0.010	0.000	0.006
	P	0.945	0.997	1.000	1.000	0.995	0.993	1.000	0.992	1.000	0.995
Puffinus lherminieri boydi	β ± SE	-0.488 ± 0.487	1.680e+01 ±4.530e+03	1.680e+01 ±4.530e+03	1.850e+01 ±7.468e+03	1.650e+01 ±2.747e+03	15.500 ±1666,410	1.741e+01 ±2.747e+03	1.700e+01 ±1.666e+03	1.875e+01 ±4.530e+03	1.580e+01 ±2.747e+03
	Z	1.001	0.004	0.004	0.002	0.006	0.009	0.006	0.010	0.004	0.006
	P	0.317	0.997	0.997	0.998	0.995	0.993	0.995	0.992	0.997	0.995

Table 10. Frequency of occurrence of both regions with the plastic type, size (mesoplastics (5mm to 2.5cm), large microplastics (1mm to 5mm), small microplastics (1µm to 1mm) and nanoplastics (1nm to 1µm)) and colour. N is the number of birds of each region. Temperate presence = 50.7% Tropical presence = 58.7%.

				Type			
Region	**N**	**Fragment %**	**Fiber %**	**Filament %**	**Microbead %**	**Rubber %**	**Ball %**
Temperate	75	15.8	97.4	—	—	—	—
Tropical	269	60.8	55.1	4.4	3.2	1.9	3.8

			Size		
Region	**N**	**Mesoplastic %**	**Large Microplastic %**	**Small Microplastic %**	**Nanoplastic %**
Temperate	75	5.3	60.5	55.3	—
Tropical	269	10.8	35.4	83.5	1.3

						Colour							
Region	**N**	**Blue %**	**Green %**	**Black %**	**Purple %**	**Transparent %**	**White %**	**Yellow %**	**Red %**	**Grey %**	**Pink %**	**Orange %**	**Multicolour %**
Temperate	75	63.2	—	5.3	2.6	26.3	—	5.3	13.2	—	—	23.7	—
Tropical	269	63.3	10.1	5.1	12.7	12.7	5.1	1.9	3.2	13.3	15.8	5.1	6.3

Table 11. Numeric frequency of both regions with the plastic type, size (mesoplastics (5mm to 2.5cm), large microplastics (1mm to 5mm), small microplastics (1µm to 1mm) and nanoplastics (1nm to 1µm)) and colour. N is the total number of plastics of each region.

				Type			
Region	**N**	**Fragment %**	**Fiber %**	**Filament %**	**Microbead %**	**Rubber %**	**Ball %**
Temperate	64	9.4	90.6	—	—	—	—
Tropical	324	49.4	43.8	2.5	1.5	0.9	1.9

			Size		
Region	**N**	**Mesoplastic %**	**Large Microplastic %**	**Small Microplastic %**	**Nanoplastic %**
Temperate	64	3.1	54.7	42.2	—
Tropical	324	5.9	26.2	67.3	0.6

						Colour							
Region	**N**	**Blue %**	**Green %**	**Black %**	**Purple %**	**Transparent %**	**White %**	**Yellow %**	**Red %**	**Grey %**	**Pink %**	**Orange %**	**Multicolour %**
Temperate	64	50.0	—	3.1	1.6	20.3	—	3.1	7.8	—	—	14.1	—
Tropical	324	49.4	5.3	4.3	6.8	6.8	3.1	0.9	1.5	7.1	8.6	2.8	3.4

Table 12. Frequency of occurrence of both foraging distributions with the plastic type, size (mesoplastics (5mm to 2.5cm), large microplastics (1mm to 5mm), small microplastics (1µm to 1mm) and nanoplastics (1nm to 1µm)) and colour. N is the number of birds of each foraging distribution. Pelagic presence = 54.7% Coastal presence = 75.2%.

				Type			
Distribution	**N**	**Fragment %**	**Fiber %**	**Filament %**	**Microbead %**	**Rubber %**	**Ball %**
Pelagic	181	54.7	42.4	3.0	4.0	2.0	4.0
Coastal	129	75.2	84.5	4.1	1.0	1.0	2.1

			Size		
Distribution	**N**	**Mesoplastic %**	**Large Microplastic %**	**Small Microplastic %**	**Nanoplastic %**
Pelagic	181	16.2	31.3	79.8	1.0
Coastal	129	3.1	49.5	76.3	1.0

						Colour							
Distribution	**N**	**Blue %**	**Green %**	**Black %**	**Purple %**	**Transparent %**	**White %**	**Yellow %**	**Red %**	**Grey %**	**Pink %**	**Orange %**	**Multicolour %**
Pelagic	181	60.6	8.1	4.0	7.1	13.1	6.1	2.0	—	8.1	22.2	1.0	—
Coastal	129	66.0	8.3	6.2	14.4	17.5	2.1	3.1	10.3	13.4	3.1	15.5	4.1

Table 13. Numeric frequency of both foraging distributions with the plastic type, colour and size (mesoplastics (5mm to 2.5cm), large microplastics (1mm to 5mm), small microplastics (1μm to 1mm) and nanoplastics (1nm to 1μm)). N is the total number of plastics of each foraging distribution.

		Type					
Distribution	N	Fragment %	Fiber %	Filament %	Microbead %	Rubber %	Ball %
Pelagic	186	61.8	31.2	1.6	2.2	1.1	2.2
Coastal	202	25.3	70.3	2.5	0.5	0.5	1.0

		Size			
Distribution	N	Mesoplastic %	Large Microplastic %	Small Microplastic %	Nanoplastic %
Pelagic	186	9.7	24.2	65.6	0.5
Coastal	202	1.5	37.1	60.9	0.5

		Colour											
Distribution	N	Blue %	Green %	Black %	Purple %	Transparent %	White %	Yellow %	Red %	Grey %	Pink %	Orange %	Multicolour %
Pelagic	186	51.1	4.8	4.8	3.8	7.5	3.8	1.1	—	5.4	13.4	0.5	3.8
Coastal	202	48.0	4.0	3.5	7.9	1.4	1.5	1.5	5.0	6.4	1.5	8.4	2.0

Table 14. Frequency of occurrence of plastic type, size (mesoplastics (5mm to 2.5cm), large microplastics (1mm to 5mm), small microplastics (1µm to 1mm) and nanoplastics (1nm to 1µm)) and colour presence in species that interact with fisheries comparing to species which do not. N is the number of birds of each group. Species that interact presence = 65.4% Species that do not interact presence = 52.1%.

		Type					
Fisheries	**N**	**Fragment %**	**Fiber %**	**Filament %**	**Microbead %**	**Rubber %**	**Ball %**
Interaction	127	48.2	69.9	1.2	3.6	—	1.2
No Interaction	217	54.9	58.4	5.3	1.8	2.7	4.4

		Size			
Fisheries	**N**	**Mesoplastic %**	**Large Microplastic %**	**Small Microplastic %**	**Nanoplastic %**
Interaction	127	19.3	51.8	63.9	1.2
No Interaction	217	2.7	31.9	88.5	0.9

		Colour										
Fisheries	**N**	**Blue %**	**Green %**	**Black %**	**Purple %**	**Transparent %**	**White %**	**Yellow %**	**Red %**	**Grey %**	**Pink %**	**Orange %**	**Multicolour % (n)**
Interaction	127	71.1	3.6	6.0	6.0	19.3	3.6	2.4	6.0	6.0	13.3	12.1	—
No Interaction	217	57.5	11.5	4.4	14.2	12.4	4.4	2.7	4.4	14.2	12.4	6.2	8.9

Table 15. Numeric frequency of plastic type, size (mesoplastics (5mm to 2.5cm), large microplastics (1mm to 5mm), small microplastics (1µm to 1mm) and nanoplastics (1nm to 1µm)) and colour presence in species that interact with fisheries comparing to species which do not. N is the total number of plastics of each group.

			Type				
Fisheries	**N**	**Fragment %**	**Fiber %**	**Filament %**	**Microbead %**	**Rubber %**	**Ball %**
Interaction	174	47.1	50.0	0.6	1.7	—	0.6
No Interaction	214	39.3	52.8	3.3	0.9	1.4	2.3

			Size		
Fisheries	**N**	**Mesoplastic %**	**Large Microplastic %**	**Small Microplastic %**	**Nanoplastic %**
Interaction	174	10.3	37.9	51.2	0.6
No Interaction	214	1.4	25.2	72.9	0.5

						Colour							
Fisheries	**N**	**Blue %**	**Green %**	**Black %**	**Purple %**	**Transparent %**	**White %**	**Yellow %**	**Red %**	**Grey %**	**Pink %**	**Orange %**	**Multicolour %**
Interaction	174	54.6	2.3	5.8	2.9	11.5	2.3	1.2	2.9	2.9	8.1	5.8	—
No Interaction	214	45.3	6.1	2.8	8.4	7.0	2.8	1.4	2.3	8.4	6.5	3.7	5.1

Table 16. Frequency of occurrence of three sizes categories with the plastic type, size (mesoplastics (5mm to 2.5cm), large microplastics (1mm to 5mm), small microplastics (1µm to 1mm) and nanoplastics (1nm to 1µm)) and colour. N is the number of birds of each group. Small birds presence = 41.9%. Medium birds presence = 65.2% Large birds 68.5%.

		Type					
Size	**N**	**Fragment %**	**Fiber %**	**Filament %**	**Microbead %**	**Rubber %**	**Ball %**
Small	129	57.4	38.9	3.7	1.9	3.7	5.6
Medium	161	65.2	68.6	1.0	2.9	1.0	1.9
Large	54	68.5	83.8	10.8	2.7	—	2.7

		Size			
Size	**N**	**Mesoplastic %**	**Large Microplastic %**	**Small Microplastic %**	**Nanoplastic %**
Small	129	3.7	20.4	87.0	—
Medium	161	15.2	48.6	69.5	1.9
Large	54	2.7	46.0	89.2	—

		Colour										
Size	**N**	**Blue %**	**Green %**	**Black %**	**Purple %**	**Transparent**	**White %**	**Yellow %**	**Red %**	**Grey %**	**Pink %**	**Orange %**	**Multicolour %**
Small	129	46.3	9.3	1.9	5.6	13.0	5.6	3.7	—	5.6	20.4	—	11.1
Medium	161	71.4	3.8	7.6	11.4	9.5	3.8	2.9	4.4	6.7	11.4	10.5	3.8
Large	54	64.9	18.9	2.7	16.2	10.8	2.7	—	7.4	29.7	5.4	16.2	—

Table 17. Numeric frequency of three sizes categories with the plastic type, size (mesoplastics (5mm to 2.5cm), large microplastics (1mm to 5mm), small microplastics (1µm to 1mm) and nanoplastics (1nm to 1µm)) and colour. N is the total number of plastics of each group.

		Type					
Size	**N**	**Fragment %**	**Fiber %**	**Filament %**	**Microbead**	**Rubber %**	**Ball %**
Small	76	51.3	38.2	2.6	1.3	2.6	4.0
Medium	227	44.9	52.0	0.4	1.3	0.4	0.9
Large	85	29.4	62.4	5.9	1.1	—	1.2

		Size			
Size	**N**	**Mesoplastic %**	**Large Microplastic %**	**Small Microplastic %**	**Nanoplastic %**
Small	76	2.6	18.4	79.0	—
Medium	227	7.9	34.8	56.4	0.9
Large	85	1.2	31.8	67.1	—

		Colour										
Size	**N**	**Blue %**	**Green %**	**Black %**	**Purple %**	**Transparent**	**White %**	**Yellow %**	**Red %**	**Grey %**	**Pink %**	**Orange %**	**Multicolour %**
Small	76	42.1	6.6	1.3	4.0	9.2	4.0	2.6	—	6.6	14.5	—	9.2
Medium	227	53.3	2.2	5.7	6.2	10.1	2.2	1.3	2.6	3.1	6.6	4.9	1.8
Large	85	45.9	8.2	2.4	7.1	5.9	2.4	—	4.7	12.9	2.4	8.2	—