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THE USE OF STRANDED AQUATIC-ASSOCIATED BIRD SURVEYS FOR PLASTIC LITTER MONITORING IN PORTUGAL

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MSc Marine Biology

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Declaração de autoria do trabalho

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

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(Marta Basto)

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Abstract

Plastics durability and persistence, combined with their high production and low rates of recovery, are causing a net accumulation of plastic debris along shorelines, surface waters, throughout the water column and in bottom sediments. Pollution by plastic debris is an increasing environmental concern all around the globe, accounting for up to 90% of marine debris. Wildlife has been severely impacted by plastic debris in coastal and aquatic environments. Macroplastics (> 20 – 100 mm) pose a health risk to several aquatic animals, including fish, turtles and birds, because of possible entanglement and ingestion. When in the environment, macroplastic debris can brittle and break through UV radiation, mechanical action and biodegradation into small sized plastic particles, designated as microplastics (1 – 5 mm), that become more bioavailable to organisms throughout the food web. However, microplastic debris can also reach aquatic environments in their original form that were manufactured for particular industrial or domestic applications, such as plastic particles used in exfoliating facial scrubs, toothpastes and resin pellets used in plastic industry. Birds are top-predators, exposed to all threats affecting these environments and this makes them ideal sentinel organisms for monitoring ecosystem changes.

Considering the knowledge gap existing in southern Europe, in particular in Portugal, about the use of stranded aquatic-associated bird surveys for plastic litter monitoring, this study tries to fill this gap by: (1) set a baseline assessment of the prevalence of plastic litter affecting multispecies populations of aquatic birds in Portugal and (2) test if species, gender, age and condition of the birds influence type and quantity of ingested plastics. In this study, the plastics accumulated in the stomachs of stranded aquatic birds collected across the Portuguese territory will be quantified and characterized.

A total of 310 birds samples comprising four species sourced from five different wildlife rescue centres (Parque Biológico de Gaia, CERVAS, CERAS, LxCRAS and RIAS) were collected and examined for the presence of plastic litter. Of these, 15.48% were found to ingest plastic litter. The average number and mass of ingested plastics was 1.62 items per individuals and 0.0771 g, respectively. Results show that aquatic-associated birds in Portugal do ingest plastic litter, as in many other countries in the world. Monitoring plastic litter ingested by aquatic-associated birds has the potential to be a part of a wide monitoring programme that can help to inform mitigation and management measures for aquatic litter.

Keywords: Plastic debris, Plastic ingestion, Aquatic birds, Multispecies, Portugal

Resumo

À medida que os plásticos se tornaram num produto indispensável no nosso quotidiano, a sua rápida produção tem sido consequentemente acompanhada por um aumento da acumulação de plásticos no meio ambiente. A durabilidade e persistência dos plásticos, combinada com a sua elevada produção e baixas taxas de recuperação, causam a acumulação de detritos plásticos ao longo das costas, águas superficiais, ao longo da coluna de água e sedimentos. A poluição por detritos plásticos é uma crescente preocupação ambiental em todo o mundo, representando cerca de 90% dos detritos marinhos. Devido ao uso excessivo e à eliminação inadequada de produtos plásticos, a vida selvagem tem sido severamente afetada pelos detritos plásticos em ambientes costeiros e aquáticos. Os macroplásticos (> 20 – 100 mm) representam uma ameaça para vários animais aquáticos, incluindo peixes, tartarugas e aves marinhas, devido à possibilidade de enredamento e ingestão. Quando no meio ambiente, os macroplásticos podem fragmentar-se através da radiação UV, ação mecânica e biodegradação em partículas plásticas mais pequenas, designadas de microplásticos (1 – 5 mm). Estas novas partículas tornam-se mais biodisponíveis para todos os organismos da cadeia alimentar e podem libertar substâncias químicas tóxicas durante o processo de degradação. As aves aquáticas são predadores expostos a todas as ameaças que afetam estes ambientes, tornando-os organismos sentinelas ideais para monitorizar mudanças nos ecossistemas.

Comparativamente ao Norte da Europa, estudos sobre o uso de aves aquáticas arrojadas para a monitorização do lixo aquático no Sul da Europa são limitados. Em Portugal, particularmente, existe apenas um estudo publicado neste tema na região do Algarve. Sendo assim, este estudo tentará preencher esta lacuna (1) estabelecendo uma avaliação base da presença de lixo plástico que afeta diversas espécies de aves associadas ao meio aquático em Portugal e (2) testando se diferentes espécies, idades, géneros e condição corporal das aves influenciam o tipo e a quantidade de detritos plásticos ingeridos. Neste estudo, os plásticos acumulados no estômago de aves aquáticas arrojadas ao longo da costa Portuguesa foram quantificados e caracterizados. Para tal, amostras das aves foram obtidas de cinco centros de recuperação de animais selvagens diferentes, nomeadamente, Parque Biológico de Gaia, Centro de Ecologia, Recuperação e Vigilância de Animais Selvagens (CERVAS), Centro de Estudos e Recuperação de Animais Selvagens (CERAS), Centro de Recuperação de Animais Silvestres de Lisboa (LxCRAS) e Centro de

Recuperação e Investigação de Animais Selvagens (RIAS). Com os dados das amostras recolhidas foram montados três conjuntos de dados diferentes. O conjunto A incluiu amostras obtidas em todos os centros de recuperação de animais selvagens e foi utilizado para estabelecer uma avaliação base da presença de lixo plástico que afeta diversas espécies de aves associadas ao meio aquático. O conjunto B incluiu apenas amostras recolhidas no centro de recuperação de animais selvagens mais a Sul do país, o RIAS, e foi utilizado para (1) testar o efeito das diferentes idades e géneros na ingestão de plásticos e para (2) determinar se existe uma correlação entre a condição corporal das aves e os detritos plásticos ingeridos. O conjunto C incluiu apenas as cegonhas-brancas (*Ciconia ciconia*) recolhidas em todos os centros de recuperação de animais selvagens que participaram neste estudo e foi utilizado para (1) testar a existência de diferenças no tipo, cor e polímero de plásticos ingeridos por amostras recolhidas no Norte e Sul do país e (2) determinar se a ingestão de plásticos aumentou nos últimos sete anos.

As aves analisadas neste estudo foram necropsiadas de acordo com os protocolos padronizados e os seus estômagos recolhidos. Os respetivos conteúdos estomacais foram lavados sobre um crivo de metal com uma malha de 1 mm, uma vez que malhas mais pequenas ficam facilmente obstruídas com o muco das paredes estomacais e restos de comida. Os plásticos foram contados e classificados segundo protocolos padronizados em plásticos industriais ou plásticos de uso quotidiano/doméstico, que posteriormente foram ainda subdivididos em folha (e.g., sacos plásticos), fios (e.g., cordas, fios de pesca), esponja, fragmentos e outros (e.g., borracha). Os plásticos foram ainda contados e classificados tendo em conta a sua cor nas seguintes categorias: branco (incluindo transparente), preto (incluindo castanho e cinzento), amarelo, verde, vermelho (incluindo cor-de-rosa), azul e mistura. Comprimento máximo (± 1 mm) de cada item plástico foi registado, sendo posteriormente contados e classificados em megaplásticos (> 100 mm), macroplásticos (> 20 – 100 mm), mesoplásticos (> 5 – 200 mm) e microplásticos (1 – 5 mm). O peso total por estômago e o peso por categoria de plástico ao valor aproximado de 0.0001 g foram devidamente registados.

No geral, um total de 310 amostras de aves de quatro espécies provenientes dos cinco centros de recuperação de animais selvagens foram recolhidos e analisados para determinar a presença de detritos plásticos. Destes, 15.48% continham detritos plásticos no estômago. O peso médio dos plásticos ingeridos foi de 0.0771 g. Das espécies amostradas, *Ciconia ciconia* apresentou a maior percentagem de ocorrência (25.86%), no

entanto *Larus fuscus* apresentou um maior número de detritos plásticos ingeridos quando comparado com as restantes espécies. Os itens plásticos encontrados foram classificados maioritariamente como plásticos de uso quotidiano/doméstico. Em relação à cor os detritos apresentaram cores variadas, tendo sido os detritos de cor branca e preta os mais abundantes.

Os resultados mostram que as aves associadas ao meio aquático em Portugal ingerem detritos plásticos, assim como em muitos outros países do mundo. Existe uma necessidade urgente de padronizar protocolos em Portugal, mas também com estudos de todo o mundo.

A monitorização da ingestão de detritos plásticos por aves associadas ao meio aquático tem potencial para ser parte de um amplo programa de monitorização que pode ajudar a encontrar medidas de mitigação e gestão para detritos presentes no meio aquático. No entanto, é necessário que as instituições governamentais desempenhem um papel ativo, enfrentando este problema através da criação de novas legislações que controlem as fontes de detritos plásticos. As indústrias de plásticos também podem desempenhar um papel importante na redução de detritos plásticos no meio ambiente, uma vez que podiam assumir responsabilidade pelo fim de vida dos seus próprios produtos plásticos, reciclando-os.

A continuação deste tipo de estudos em Portugal é crucial para que se possa obter resultados baseados num maior número de amostras de diferentes espécies e para podermos identificar quais as espécies mais indicadas para monitorizar a presença destes detritos no meio ambiente. Uma vez que a ingestão de plásticos por aves ou outros animais aquáticos têm potenciais efeitos nocivos, torna-se urgente avaliar os efeitos sobre a saúde, particularmente no caso de espécies ameaçadas.

Palavras-chave: Detritos plásticos, Ingestão de plásticos, Aves aquáticas, Portugal

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List of Abbreviations

WRC – Wildlife rescue centre

PBGaia – Parque Biológico de Gaia

CERVAS – Centro de Ecologia, Recuperação e Vigilância de Animais Selvagens

CERAS – Centro de Estudos e Recuperação de Animais Selvagens

LxCRAS – Centro de Recuperação de Animais Silvestres de Lisboa

RIAS – Centro de Recuperação e Investigação de Animais Selvagens

ATR – Attenuated total reflectance

CC – *Ciconia ciconia*

LF – *Larus fuscus*

LM – *Larus michahellis*

MB – *Morus bassanus*

PP – Polypropylene

PE – Polyethylene

PE-HD – High-density polyethylene

PE-LD – Low-density polyethylene

PVC – Polyvinyl chloride

PUR – Polyurethane

PET – Polyethylene terephthalate

PVA – Polyvinyl alcohol

PS – Polystyrene

PDMS – Polydimethylsiloxane

PA – Polyamide

POPs – Persistent organic pollutants

UV – Ultraviolet radiation

BPA – Bisphenol A

PBDEs – Polybrominate diphenyl ethers

PCBs – Polychlorinated biphenyl

DDT – Dichlorodiphenyltrichlorethane

OSPAR – Oslo/Paris Convention for the Protection of the Marine Environment of the North-East Atlantic

MSFD – Marine Strategy Framework Directive

1. Introduction

1.1. Worldwide plastic production

Plastics benefits, including its versatility, resistance and durability to degradation (Avio *et al.*, 2016), led to the current period of human history referred as the Plastic Age (Yarsley and Couzens, 1945). However, the extreme use and inappropriate disposal of plastic products are leading to a visible accumulation of plastic debris (Barnes *et al.*, 2009).

Plastics are composed of more than twenty families of polymers, six of which are referred to as "big six", and include polypropylene (PP), high- and low-density polyethylene (PE-HD and PE-LD), polyvinyl chloride (PVC), polyurethane (PUR), polyethylene terephthalate (PET) and polystyrene (PS), which together account for over 90% of European plastic production (PlasticsEurope, 2015). Because only a small portion of plastic is recycled and because plastic debris fragments and degrades at a very slow rate, these polymers tend to accumulate the most in all types of environments, especially in aquatic ones (Andrady, 2011; Dehaut *et al.*, 2016; Engler, 2012).

Pollution by plastic debris is an increasing environmental concern all around the globe. Since the 1950s, global plastic production is increasing exponentially with a current doubling time of 11 years, going from 1.7 million tonnes in 1950 to 335 million tonnes in 2016 (Figure 1.1; Law, 2016; PlasticsEurope, 2013, 2015, 2016, 2017; Wilcox *et al.*, 2015).

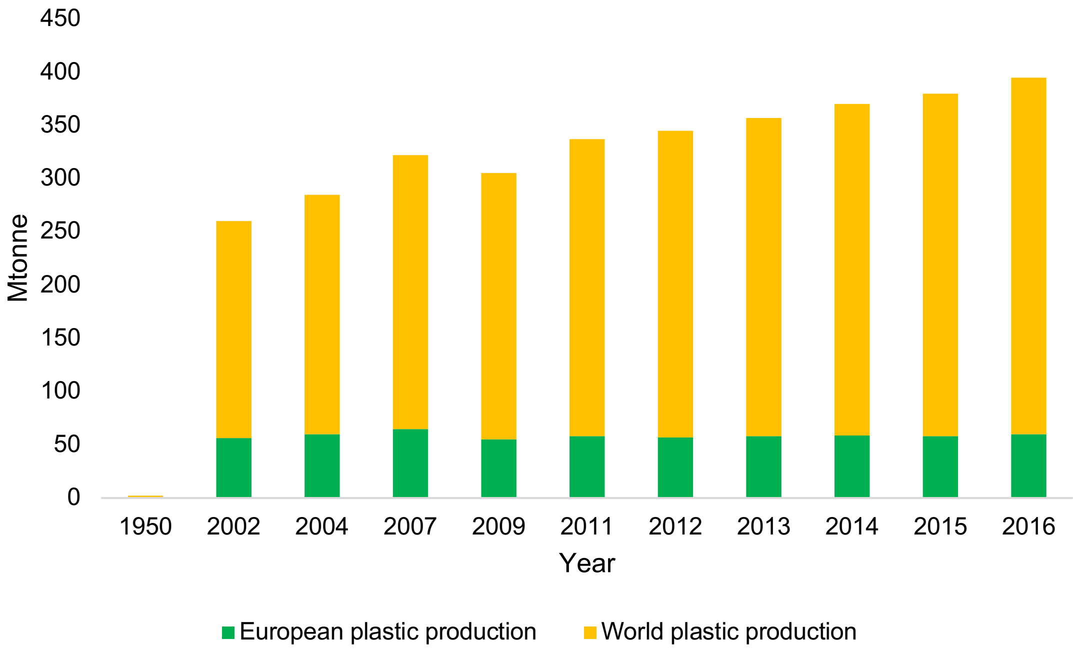

Figure 1.1: Worldwide and European plastic production between 1950 and 2016 (adapted from PlasticsEurope, 2013, 2015, 2016, 2017).

In 2016, right after the largest plastic producer (China, contributing with 29% of the world's total production), Europe was the second largest plastic producer, accounting for 19% of the world's total production, corresponding to 60 million tonnes of plastic produced in that year (PlasticsEurope, 2017). In the same year, the European plastic industry gave direct employment to over 1.5 million people, generating almost 30 billion euros to public finances and welfare (PlasticsEurope, 2017), approximately 2.5 billion euros more than the previous year. The largest plastic producers were the packaging sector (39.9%) followed by building and construction (19.7%), other market sectors (16.7%; includes appliances, mechanical engineering, furniture, medical, etc.), automotive (10%), electrical and electronic (6.2%), household, leisure and sports (4.2%) and agriculture (3.3%; PlasticsEurope, 2017).

Since plastics became a product present in our daily life, its rapid growth in production has been accompanied by a consequent increase in the concentration of plastics in marine and coastal environments, such as beaches, waterways, estuaries, lakes, coral reefs, the open as well as deep sea (Barnes *et al.*, 2009; Cózar *et al.*, 2014; Donohue *et al.*, 2001; Free *et al.*, 2014; Lima *et al.*, 2014; Moore and Phillips, 2011; Moore *et al.*, 2011; Thompson *et al.*, 2004; Van Cauwenberghe *et al.*, 2013).

1.2. Classification of plastics

Plastic debris can be classified according to size, origin, shape and composition (Driedger *et al.*, 2015). The most commonly used size categories include mega- (> 100 mm), macro- (>20 – 100 mm), meso- (5 to 20 mm) and microplastics (< 5 mm; Romeo *et al.*, 2015; Ryan *et al.*, 2009; Sanchez *et al.*, 2014); however a globally accepted definition does not exist (Provencher *et al.*, 2017), and thus finding a standard classification of size categories has been a recent research priority (Morét-Ferguson *et al.*, 2010; Vegter *et al.*, 2014). Provencher *et al.* (2017) advocates the use of the size categories proposed by Barnes *et al.* (2009) as the most relevant and applicable as includes extra-large sizes of plastics that are usually ingested by marine megafauna (i.e., marine mammals, turtles and seabirds). This classification includes megaplastics (> 100 mm), macroplastics (> 20 – 100 mm), mesoplastics (> 5 – 20 mm) and microplastics (1 - 5 mm; Barnes *et al.*, 2009).

Plastic debris can be classified as either primary or secondary. Primary plastics are those that, when collected, are in their original or close-to-original form, such as bottle caps, cigarette butts, microbeads, plastic pellets or synthetic clothing fibres (Chang, 2015; Mato *et al.*, 2001; Napper *et al.*, 2015; van Wezel *et al.*, 2015; Wagner *et al.*, 2014). Secondary plastics results from the breakdown of primary debris through several environmental degradation processes (Browne *et al.*, 2007; Cole *et al.*, 2011; Shah *et al.*, 2008; Thompson *et al.*, 2004; Wagner *et al.*, 2014). In addition to the recognizable plastic objects, plastic debris can exhibit a different range of shapes and are thus classified in sheetlike (i.e., plastic bags, foils and clingfilm), threadlike (i.e., remains of ropes, nets, nylon line, packaging straps, etc.), foam (i.e., foamed polystyrene cups, packaging, construction foams), fragments (i.e., bottles, boxes, toys, toothbrushes, etc.) and others (i.e., cigarette filters, rubber, elastics, etc.; Van Franeker *et al.*, 2011).

In terms of composition, there are many typologies of plastic polymers and additives that can be combined in objects with specific properties and characteristics (Avio *et al.*, 2016). The most common polymers are polyethylene (PE), polypropylene (PP), polystyrene (PS), polyvinylchloride (PVC), polyamide (PA), polyethylene terephthalate (PET) and polyvinyl alcohol (PVA; Avio *et al.*, 2016). When in the ocean, their consequence in the environment will depend on the polymer density (Table 1.1), which will determine their buoyancy and consequently their position in the water column and their potential to affect biota (Wright *et al.*, 2013). Polymers denser than seawater (i.e., PVC) will tend to sink, while polymers with lower density (i.e., PE and PP) will tend to float in the water column (Avio *et al.*, 2016; Driedger *et al.*, 2015). Furthermore, buoyancy can be affected by processes such as biofouling and the colonization of organisms on plastics surface that increases the weight of particles, thus accelerating their sinking on bottom sediments (Lobelle and Cunliffe, 2011; Ye and Andrady, 1991); in addition, other factors such as degradation, fragmentation and leaching of additives can also interfere with plastic density, and hence, alter plastics distribution in the water column (Avio *et al.*, 2016).

Table 1.1: Density range of the most common polymers of environmental relevance (Avio *et al.*, 2016).

Matrix	Density (g/cm^3)
Distilled water	1
Seawater	1.025
Polyethylene (PE)	0.93 – 0.98
Polypropylene (PP)	0.89 – 0.91
Polystyrene (PS)	1.04 – 1.11
Polyvinylchloride (PVC)	1.20 – 1.45
Polyamide (PA)	1.13 – 1.5
Polyethylene terephthalate (PET)	1.38 – 1.39
Polyvinyl alcohol (PVA)	1.19 – 1.35

1.3. Origin, presence and impacts of plastic debris in the aquatic environment

Aquatic litter (or aquatic debris) comprises any manufactured or processed solid material that was discarded or transported into any aquatic environment, as well as glass, metals, paper, textiles, wood, rubber and plastics. Several of these materials may be promptly biodegradable (i.e., paper, wood or natural fibres), while others remain for long periods of time in the marine or any other aquatic environment. When compared to other materials, plastics are unique since they are both persistent (resistant to biodegradation) and, because of their light weight, readily transportable by wind and water (Law, 2016).

In 1972, the first observations of microplastic pollution in marine ecosystems was recorded (Carpenter *et al.*, 1972). Recently, it was estimated that at least 8 million tonnes of plastic enter in the oceans every year (Jambeck *et al.*, 2015), comprising 90% of the marine litter (Barnes *et al.*, 2009; Derraik, 2002; Galgani *et al.*, 2015; Rios *et al.*, 2007). Between 7000 and 250,000 tonnes of plastics are estimated to occur on surface waters (Cózar *et al.*, 2014; Eriksen *et al.*, 2014), in the water column (Lattin *et al.*, 2004) and in seabed sediments (Fischer *et al.*, 2015; Fries *et al.*, 2013; Van Cauwenberghe *et al.*, 2013). High concentrations of floating plastic debris have been reported in central areas of North Atlantic (Law *et al.*, 2010) and Pacific Oceans (Eriksen *et al.*, 2013b; Goldstein *et al.*, 2012), indicating that plastic pollution can reach even the most remote areas of the planet (Table 1.2; Cózar *et al.*, 2014). These models predict that these large-scale vortices act as conveyor belts, collecting the floating plastic debris released from the continents and accumulating it into central convergence zones (Cózar *et al.*, 2014).

Table 1.2: Average concentration of floating plastic debris reported around the globe.

Location	Region	Average concentration	Plastic type	Reference
Atlantic Ocean	North Sea	1.6 ± 0.4 items/Ha	Macroplastics	Galgani *et al.* (2000)
	Portuguese coast	0.02 – 0.04 items/m^3	Microplastics	Frias *et al.* (2014)
	Celtic sea	2.46 items/m^3	Macroplastics and microplastics	Lusher *et al.* (2014)
Pacific Ocean	North Pacific Centre gyre	334.271 items/Km^2	Macroplastics and microplastics	Moore *et al.* (2001)
	South Pacific subtropical gyre	26,898 items/Km^2	Macroplastics and microplastics	Eriksen *et al.* (2013b)
	East China Sea	0.167 ± 0.138 items/m^3	-	Moore *et al.* (2002)
Mediterranean Sea	-	0.243 items/m^2	-	Cózar *et al.* (2015)
The United States	Laurentian Great Lakes	43,000 items/Km^2	Macroplastics and microplastics	Eriksen *et al.* (2013a)

However, not only marine environments are contaminated by plastics; in Singapore, microplastics were extracted from seven intertidal mangrove sediments, where microplastics concentrations ranged from 12.0 to 62.7 particles per Kg of dry sediment, fibres were the most common plastic shape found and PE and PP the polymer types encountered (Nor and Obbard, 2014). The presence of microplastics, more specifically PE microbeads (0.40 – 2.16 mm in diameter), were reported in the sediments of the St. Laurence River with a mean density of 13 832 (± 13 667) microbeads/m^2

(Castañeda *et al.*, 2014). In South Africa, five estuaries along the Durban coastline were analysed for the presence of plastics in their sediments. Plastics were found in all study sites, although three (Durban harbour with 159.9 ± 271.2 particles per 500 mL, Isipingo with 47.6 ± 22.8 particles per 500 mL and uMgeni with 41.7 ± 23.0 particles per 500 mL) presented higher concentrations of plastics compared to the other two (Mdloti with 19.9 ± 16.2 particles per 500 mL and iLovu with 13.7 ± 5.6 particles per 500 mL), being fragments and fibres the main plastic shapes found (Naidoo *et al.*, 2015). In Italy, the Lagoon of Venice was a target of study for the identification, distribution and abundance of microplastic particles, where total abundances ranged from 2175 to 672 particles per Kg of dry weight, with higher concentrations observed mostly in landward sites. PE and PP were the most frequent polymers found (Vianello *et al.*, 2013).

Portugal is no exception, plastics are the most predominant type of floating debris in our offshore waters (Barnes *et al.*, 2009; Dixon and Dixon, 1983; Thiel *et al.*, 2011; Thiel *et al.*, 2013) and has been described as the main type of marine debris covering the sea bottom and submarine canyons, as well as deposited on beaches (Mordecai *et al.*, 2011; Neves, 2013; OSPAR, 2007). For example, microplastic debris were found in almost 56% of sediment samples from the southern Portuguese shelf waters, being the majority microfibers, identified as rayon fibres, and fragments, identified as PP (Frias *et al.*, 2016). Along the western coast of Portugal, sediment was sampled in five beaches and a total amount of 17799 plastic debris were collected with an average density of 185.1 items/m^2. The plastic particles size ranged from 50 µm to 20 cm, but the majority were microplastics (< 5 mm; Martins and Sobral, 2011).

The main inputs of plastics into the sea derive from beaches and land-based sources, such as rivers, storm water runoff, wastewater discharges or transport of land litter by wind (Ryan *et al.*, 2009). Marine activities also contribute by introducing materials that are lost by professional and recreational fishing, and debris dumped by commercial, cruise or private ships (Figure 1.2; Cooper and Corcoran, 2010). In Continental Portugal, land sources comprise river discharges and coastal urban centres while marine sources include fisheries and recreational maritime activities (Neves, 2013), commercial vessels and cruise ships (Martins and Sobral, 2011).

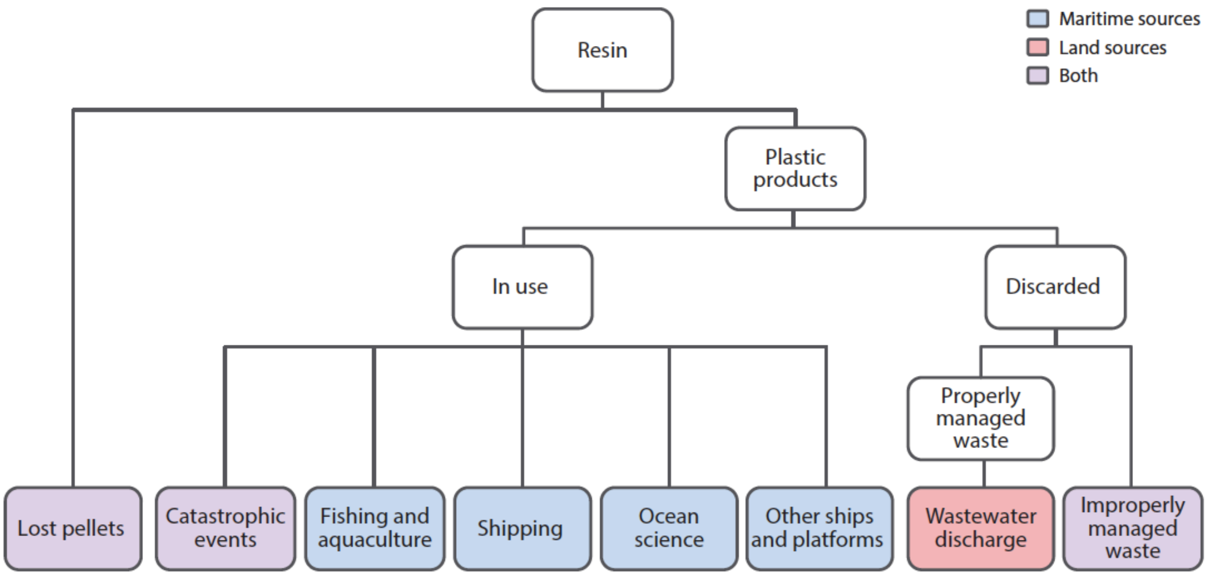

Figure 1.2: Flow chart describing inputs of plastics into the marine environment, beginning with the manufacture of common plastic resins in the form of industrial pellets. The lowest level shows direct sources to the marine environment; blue shading indicates sources from marine activities, red indicates sources from land activities and purple indicates sources from either maritime or land activities (Law, 2016).

Plastic accumulation in aquatic environments in general has several consequences. From an economic perspective, aquatic litter can interfere with subsistence fishing practices, causing changes in those practices and potential income (Nash, 1992). Ecotourism can be negatively affected as well, by creating unappealing coastal land and seascapes (Gregory, 1999; Jang *et al.*, 2014). Plastic contamination is a major cost for local and regional governments, since clean-up actions are extremely costly, reaching millions of dollars a year (Mouat *et al.*, 2010; UNEP, 2014; Vegter *et al.*, 2014).

Because of possible entanglement and ingestion, plastics pose a health risk to a variety of aquatic animals, including fish, turtles and birds (Table 1.3 and Table 1.4; Boerger *et al.*, 2010; Codina-García *et al.*, 2013; Gregory, 2009; Laist, 1997; Sheavly and Register, 2007). Entanglement can cause injuries, drowning, suffocation, reduced ability to predate and increase the probability of being caught (Derraik, 2002; Gall and Thompson, 2015; Laist, 1997). The most common encounter material reported are fishing materials, originated from fishing activities or cargo ships (Gilardi *et al.*, 2010; Kiessling, 2003; Macfadyen *et al.*, 2009), being more than 6.4 tonnes of fishing gear abandoned or lost each year in the sea (Macfadyen *et al.*, 2009). Most nets are made from synthetic materials, since they are cheaper, more durable and more lightweight, however, when eventually unusable and lost, they continue to indiscriminately entangle marine organisms (Gilardi *et al.*, 2010). Sea turtles are probably the most susceptible species to "ghost netting" as they often use floating objects for either shelter to avoid predation or as foraging stations (White, 2006), showing that entanglement incidence for certain species can be linked to behavioural strategies (Derraik, 2002). Ingestion of plastic debris are physical hazards to the organism that ingest them (Fendall and Sewell, 2009), since they may cause bleeding, blockage of the digestive tract, ulcers or perforations and produce a deceptive satiation feeling, causing the organism not to feed, and consequently leading to starvation (Derraik, 2002; Ryan, 1988a; Ryan, 1988b; Wright *et al.*, 2013). In some species, ingestion is reported in over 80% of a population sampled (i.e., Murray and Cowie, 2011; Van Franeker *et al.*, 2011), for example, 95% of 1,295 beached seabird (Northern Fulmar, *Fulmarus glacialis*) carcasses in the North Sea contained plastic in their stomach contents (Van Franeker *et al.*, 2011).

Table 1.3: Studies demonstrating evidence of plastic debris entanglement by marine organisms.

Species	Location	Entanglement rate (%)	Reference
Northern gannets	Spanish Iberia and Mauritania	0.93%	Rodríguez *et al.* (2013)
Seals, sea lions, gulls, fulmars and turtles	United States	-	Moore *et al.* (2009)
Gorgonians	Azores, Portugal	-	Pham *et al.* (2013)
New Zealand fur seals	Cape Gantheaume, Kangaroo Island	0.73%	Page *et al.* (2004)
Australian sea lions	Seal Bay, Kangaroo Island	0.83%	Page *et al.* (2004)

Table 1.4: Studies demonstrating evidence of plastic ingestion by marine wildlife.

Species	Location	Percentage frequency of occurrence (%)	Reference
Seabirds	Ireland	0%	Acampora *et al.* (2016)
Seabirds	Catalan coast, Mediterranean	96%	Codina-García *et al.* (2013)
Sea turtles	Mediterranean	37%	Revelles *et al.* (2007)
Fishes	English Channel	36.5%	Lusher *et al.* (2013)
Blue mussel	North Sea, Germany	-	De Witte *et al.* (2014)
True's beaked whales	North and West coast of Ireland	85%	Lusher *et al.* (2015)
Zooplankton	Portuguese coastal waters	61%	Frias *et al.* (2014)

Once in the environment, plastic debris get exposed to ultraviolet (UV) radiation, mechanical weathering and biodegradation, and they brittle and brake into smaller particles (Andrady, 2011). These particles can release toxic chemicals during the degradation process that were initially incorporated during manufacturing or adsorbed to their surfaces while in the environment (Driedger *et al.*, 2015). Some of these chemicals includes persistent organic pollutants (POPs), such as phthalates, nolyphenols, bisphenol A (BPA), polybrominated diphenyl ethers (PBDEs; Bittner *et al.*, 2014; Mato *et al.*, 2001; Rios *et al.*, 2010; Teuten *et al.*, 2007; Zarfl and Matthies, 2010), and heavy metals (Ashton *et al.*, 2010; Cheng *et al.*, 2010; Holmes *et al.*, 2012; Nakashima *et al.*, 2011), which can disrupt endocrine functions and cause harmful reproductive and developmental effects in aquatic animals (Meeker *et al.*, 2009). The biodegradation of these POPs has been shown to slow down when these are adsorbed on plastics, increasing their persistence in the environment (Teuten *et al.*, 2009).In the Portuguese coast, PCBs (0.02 – 15.56 ng g^{-1}) and DDT (0.16 – 4.5 ng g^{-1}) have been found on plastic pellets (Frias *et al.*, 2010). As plastic particles become smaller, they also become available for organisms throughout the food web (Andrady, 2011; Boerger *et al.*, 2010; Fossi *et al.*, 2012; Teuten *et al.*, 2009). Although direct transfer of plastic-sorbed toxins to organisms through oral ingestion has been shown (i.e., Rochman *et al.*, 2013; Ryan *et al.*, 1988), how and if this also occurs in humans is still largely unknown (Driedger *et al.*, 2015). Plastic debris can also transport nonnative species (Barnes, 2002; Barnes *et al.*, 2009; Gregory, 2009) and be colonized my microbes including possible pathogens (Wagner *et al.*, 2014; Zettler *et al.*, 2013).

The accumulation of sinking plastic debris and dragging of fish nets in the littoral zones, may disrupt bottom sediments, displace or smother infauna, eventually affecting the structure and functioning of benthic communities (Goldberg, 1994). In coastal areas, plastic accumulation can avert recreational usage, pose a threat to swimmers and divers and carry a risk of cuts or abrasion injuries to beach-goers (Sheavly and Register, 2007). Since tourists use beach cleanliness as a dominant factor in selecting recreational destinations, plastic debris can reduce income generated from tourism due to forced beach closers (Jeftić *et al.*, 2009). Macroplastic debris represents a navigational and structural hazard to shipping vessels and small marine vehicles, including burnt out water pumps and entangled propellers (Mouat *et al.*, 2010). Abandoned fish nets and other plastic gear may trap commercial fish unintentionally, hence removing them from the pool available for harvest (Gregory, 2009).

1.4. Use of aquatic birds as indicators of plastic litter

Because plastic litter present in aquatic environments can be positively buoyant, aquatic predators are susceptible to ingest plastic debris while feeding on surface waters (Baulch and Perry, 2014). Although plastic ingestion and entanglement has been documented in over 100 species of aquatic animals (Laist, 1997), aquatic birds, more specifically seabirds, have been recognised as a useful indicator, or sentinel species, for aquatic pollution within both scientific literature and though existing policy (OSPAR, 2008; Van Franeker *et al.*, 2011). This recognition as valuable indicators is firstly because they are wide-ranging foragers, they occupy a high-trophic position (predators), they breed at specific locations that are relatively easy to access for study purposes and show large scale distributions (Burger and Gochfeld, 2004; Furness and Camphuysen, 1997; Piatt *et al.*, 2007; Provencher *et al.*, 2014a; Robards *et al.*, 1997; Ryan, 2008). Secondly, several species feed mostly on prey that may also be consumed by humans, such as numerous epipelagic fish and cephalopods, emphasizing the potential usefulness of aquatic birds as sentinels of aquatic contamination (Roscales *et al.*, 2011). Lastly, aquatic birds can often be retrieved dead on beaches and are thus sampled with relatively little collection effort (Van Franeker *et al.*, 2011).

At least 50% of all aquatic bird species are known to be affected by aquatic plastic litter (Kühn *et al.*, 2015), and it has been predicted that by 2050, 99% of all aquatic bird species and 95% of individuals will have ingested plastic debris (Wilcox *et al.*, 2015). In waters from the North Hemisphere and around South America, ingestion of plastic by aquatic birds and its effects are particularly well documented (i.e., Copello and Quintana, 2003; Ryan, 1989; Van Franeker *et al.*, 2011; Yamashita *et al.*, 2011). There is emerging evidence of negative impacts on both bird body condition and reproduction. In addition, plastic ingestion can lead to transmission of toxic chemicals and, eventually, increase mortality rates (Lavers *et al.*, 2014; Spear *et al.*, 1995; Tanaka *et al.*, 2013). Therefore, monitoring the incidence of ingestion and types of plastic ingested is of major importance, since it not only provides data on affected species and a baseline for long-term trends, as it is a cost effective mean to monitor plastic pollution levels in aquatic environments (Ryan *et al.*, 2009; Tourinho *et al.*, 2010). For example, the Northern Fulmar (*Fulmarus glacialis*) is used by both OSPAR (Oslo/Paris Convention for the Protection of the Marine Environment of the North-East Atlantic) and the European MSFD (Marine Strategy Framework Directive) for monitoring plastic pollution and support international legislation aiming at reducing aquatic litter in the North Sea (E.C, 2008, 2010; OSPAR, 2008). Although selecting an individual species to monitor plastic pollution is of major importance, a multispecies approach is crucial to understand the factors that influence plastic litter ingestion, variation in composition amounts and trends among different species and to determine an alternative species for use in a monitoring program (Acampora *et al.*, 2016).

Plastic ingestion in aquatic birds tend to increase with plastic exposure, i.e., if more plastics are introduced in aquatic environments, it is expected that ingestion rates will increase proportionally. For example, fulmars from the North Sea or from California contained more plastic debris than fulmars from presumably cleaner Artic breading locations (Van Franeker, 1985). However, there are other factors that can influence plastic ingestion as well; the colour of the plastics ingested can give information on how organisms may select plastics from the environment. Additionally, the size of plastics can influence the risk of being ingested by different organisms with different foraging strategies (Moser and Lee, 1992; Santos *et al.*, 2016). Because birds detect prey from above, it has been shown that they ingest more plastics items that contrast with ocean background, such as light coloured plastics (Santos *et al.*, 2016). Albeit, to evaluate selectivity, organismal data must be paired with environmental assessments on the availability of different coloured plastics in the environment, information that is lacking in many regions (Provencher *et al.*, 2017). Colour might also be related with higher exposure to several chemicals (Christie, 1994; Endo *et al.*, 2005). Aquatic birds' contamination is also expected to increase according to feeding techniques, with filter feeders being more contaminated than single-prey catchers, because filter feeders do not target specific items, and surface feeders being more contaminated than divers because plastics are mainly at the surface (Reisser *et al.*, 2015). The ingestion of plastic debris by adults might be an indicative of individuals' large range and distribution if ingested plastic debris accumulates in the gastrointestinal tract of individuals. In addition, breeding stage can also influence adults' debris loads since adults have the capacity to regurgitate food items that may contain plastics to young chicks (inter-generational transfer), which leads to a steady decrease in adults' plastic accumulation during breeding season (Carey, 2011). Gender differences in ingestion can be due to strong variations in parental duties, as for instance incubation performed mainly by females and general duties carried out by males (Bochenski and Jerzak, 2006; Wuczyński, 2012).

As birds ingest plastics and they accumulate them in their stomachs, plastics compete with food for space. So, measuring the mass of accumulated plastic litter in aquatic birds is possibly the most important metric from a biological perspective, because the mass of plastic debris holds information on the volume of plastics in an individual (Provencher *et al.*, 2017). Several birds also rely on reducing the ratio between body mass and wing size (wing-loading) for flight and diving, thus a plastic-loaded bird will be in disadvantage since it becomes heavier (Provencher *et al.*, 2017).

1.5.Objectives

Comparatively to northern Europe (i.e., Bond *et al.*, 2014; Kühn and van Franeker, 2012; Provencher *et al.*, 2014a; Provencher *et al.*, 2014b; Van Franeker, 1985; Van Franeker *et al.*, 2011), in southern European countries, attempts to monitor plastic litter in aquatic birds have been so far limited (i.e., Codina-García *et al.*, 2013). Particularly in Portugal, the only published information concerning this theme, is restricted to the Algarve (Nicastro *et al.*, 2018). Therefore, the general aims of this thesis are to (1) set a baseline assessment of the prevalence of plastic litter affecting multispecies population of aquatic-associated birds in Portugal and (2) test if species, age, gender and condition of the birds influences the type, quantity, colour and polymer of ingested plastics. In this study, aquatic-associated birds were considered all birds that leave on or around water (Veldman *et al.*, 2013).

1.6. Species of interest

1.6.1. *Alca torda* (Razorbill; Linnaeus, 1758)

The species *Alca torda* belongs to the order Charadriiformes, Family Alcidae (BirdLifeInternational, 2018).

The razorbill occurs in the north Atlantic, being Britain an important location of this species (Gooders *et al.*, 1996). They breed on islands, rocky shores and cliffs on northern Atlantic coasts, in eastern North America and in western Europe from north-west Russia to north-west France (Nettleship, 1996). In Portugal, occurs as a migratory and wintering species throughout all continental coast (Meirinho *et al.*, 2014).

This species inhabits rocky sea coasts (Nettleship, 1996), only coming ashore to breed (Bruun *et al.*, 1995), nesting on cliff ledges and among boulders (Nettleship, 1996). They are pursuit divers that are capable of propelling themselves through the water with its wings and dive to a maximum depth of 120 m (BirdLifeInternational, 2018). However, razorbills mostly forage near the surface (BirdLifeInternational, 2018). This species was characterized as being pelagic (Bruun *et al.*, 1995). In Portugal, this species seems to feed mainly on European pilchard (*Sardina pilchardus*), but there is also evidence of ingesting European anchovy (*Engraulis encrasicolus*) and species from the Family Ammodytidae (Beja, 1989).

According to the IUCN Red List of Threatened Species (BirdLifeInternational, 2018), this species is classified as Near Threatened (NT).

1.6.2. *Ardea cinerea* (Grey Heron; Linnaeus, 1758)

The grey heron (*Ardea cinerea*) belongs to the Order Ciconiiformes, Family Ardeidae (BirdLifeInternational, 2018).

Individuals of this species can be found throughout most of temperate Europe and extends through Russia to Japan, reaching south through China to India and can also be found in parts of Africa and in Madagascar (Gooders *et al.*, 1982).

This species occurs in freshwater habitats, such as rivers lakes, ponds and reservoirs (Gooders *et al.*, 1982). It breeds either solitarily or in colonies, designated as heronries, in woodland close to water (Svensson *et al.*, 1999). Grey heron's diet consists mainly on fish (Gooders *et al.*, 1982), which they haunt by patiently stand completely still at the side of the water and strike rapidly when a fish comes into range (Svensson *et al.*, 1999). Amphibians, small mammals, birds and invertebrates may also be part of their diet (Gooders *et al.*, 1982)

According to the IUCN Red List of Threatened Species (BirdLifeInternational, 2018), this species is classified as Least Concern (LC).

1.6.3. *Bubulcus ibis* (Cattle Egret; Linnaeus, 1758)

Bubulbus ibis belongs to the Order Ciconiiformes, Family Ardeidae (BirdLifeInternational, 2018).

This species has a large range and nests in North and South America, Africa, Europe, Asia and Australia (Kushlan and Hancock, 2005). The cattle egret can be found in open grassy areas, such as meadows, freshwater swamps (del Hoyo *et al.*, 1992), pastures, marshes (Kushlan and Hancock, 2005) and flood plains (Hancock and Kushlan, 1984), however has a preference for freshwater (Marchant and Higgins, 1990) and is rarely found near marine environments (del Hoyo *et al.*, 1992). Most cattle egret populations are partially migratory; whether a population migrates or not depends on climate and food availability (del Hoyo *et al.*, 1992). This species of egret is an opportunistic feeder, feeding on a variety of insects, spiders, frogs and worms (Brown *et al.*, 1982; del Hoyo *et al.*, 1992; Hancock and Kushlan, 1984).

According to the IUCN Red List of Threatened Species (BirdLifeInternational, 2018), this species is classified as Least Concern (LC).

1.6.4. *Ciconia ciconia* (White Stork; Linnaeus, 1758)

The species *Ciconia ciconia* belongs to the Order Ciconiiformes, Family Ciconidae (BirdLifeInternational, 2018) and is one of the two species that occurs in Portugal (EquipaAtlas, 2008).

Its distribution area extends practically throughout Continental Europe, the Middle East, North and South Africa (Snow and Perrins, 1998). In Europe, there are two populations of *C. ciconia*, a western population that migrates through the Strait of Gibraltar, wintering in West and Central Africa, and an eastern population, which migrates across the Bosporus Strait and though Israel, wintering in Central and South Africa (Araújo, 1998). In Portugal, its distribution extends almost throughout the hole national territory, except for Minho, Douro Litoral and Serra da Estrela massif (EquipaAtlas, 2008). It is a migratory and dispersive species (Snow and Perrins, 1998), but there has been an increase in the wintering population in the European continent (Catry *et al.*, 2010; Rosa *et al.*, 2009).

This is an opportunistic species that feeds depending on the availability, alone or in flocks, of earthworms, insects, fish, amphibians and small mammals caught mainly while walking or running with the head and the beak pointed down, often with some wing beats (Snow and Perrins, 1998; Tryjanowski and Kuzniak, 2002). Storks normally feed on the surrounding nesting grounds, however they can do 3 to 5 kilometres to feed and in areas with concentrated tusks, they can fly long distances (Snow and Perrins, 1998). Additionally, it has been shown that this species uses landfills and sanitary landfills as feeding sites throughout almost all its distribution (Ciach and Kruszyk, 2010; Donázar, 1992; Tortosa *et al.*, 2002).

According to the IUCN Red List of Threatened Species (BirdLifeInternational, 2018), this species is classified as Least Concern (LC).

1.6.5. *Ciconia nigra* (Black Stork; Linnaeus, 1758)

The species *Ciconia nigra* belongs to the Order Ciconiiformes, Family Ciconidae (BirdLifeInternational, 2018).

The black stork breeds across the Palaearctic, being widespread across much of central and eastern Europe during summer, with a patchier distribution in western Europe and a partially resident population in Spain and Portugal (del Hoyo *et al.*, 1992). This species overwinters in the Iberian Peninsula (Cano Alonso, 2006), Middle East (Van Den Bossche, 1996), Africa and also from western Pakistan, through northern India, to south-east Asia and eastern China (Bobek *et al.*, 2008; del Hoyo *et al.*, 1992).

The species inhabits old, undisturbed, open forests (del Hoyo *et al.*, 1992; Snow and Perrins, 1998) from sea-level up to mountainous regions (Hancock *et al.*, 1992). It forages in shallow streams, pools, marshes (del Hoyo *et al.*, 1992), swampy patches (Snow and Perrins, 1998), damp meadows (Hancock *et al.*, 1992), flood-plains, pools in dry riverbeds (Hockey *et al.*, 2005) and sporadically grasslands (del Hoyo *et al.*, 1992) especially where there are stands of reed or long grass (Brown *et al.*, 1982). This species feeds mostly on fish, although it may also feed on insects, amphibians, snails, crabs, small reptiles, mammals and birds (del Hoyo *et al.*, 1992). It forages mostly in shallow waters where they stalk its prey, catching them with a quick stab of the beak (del Hoyo *et al.*, 1992).

According to the IUCN Red List of Threatened Species (BirdLifeInternational, 2018), this species is classified as Least Concern (LC).

1.6.6. *Egretta garzetta* (Little Egret; Linnaeus, 1766)

The little egret (*Egretta garzetta*) belongs to the Order Ciconiiformes, Family Ardeidae (BirdLifeInternational, 2018).

Specimens can be found throughout southern Europe, southern Asia and Africa, but smaller populations can also be found in Australia (Kushlan and Hancock, 2005). Individuals are never far from water, being usually found in large wetland areas, such as mudflats and marshland, but it can also be found foraging in tidal estuaries or small streams (Kushlan and Hancock, 2005).

This species is a highly opportunistic feeder (Kushlan and Hancock, 2005), feeding mostly on small fish (del Hoyo *et al.*, 1992; Kushlan and Hancock, 2005), terrestrial and aquatic insects (i.e., beetles, dragonfly larvae, mole crickets and crickets; Kushlan and Hancock, 2005) and crustaceans (del Hoyo *et al.*, 1992), as well as amphibians, molluscs (snails and bivalves; del Hoyo *et al.*, 1992; Kushlan and Hancock, 2005), spiders, worms, reptiles and small birds (del Hoyo *et al.*, 1992).

According to the IUCN Red List of Threatened Species (BirdLifeInternational, 2018), this species is classified as Least Concern (LC).

1.6.7. *Gavia stellata* (Red-throated Loon; Pontoppidan; 1763)

Gavia stellata belongs to the Order Gaviiformes, Family Gavidae (BirdLifeInternational, 2018).

The species is migratory, breeding north of 50° N and far into high Artic, and wintering mainly along the north coast of Atlantic and Pacific Oceans, on Great Lakes, and Black, Caspian and Mediterranean Seas (Carboneras *et al.*, 2018). It breeds on fresh water pools or lakes in open moorland, blanket bogs (del Hoyo *et al.*, 1992) or open and wet peatland areas (Campbell, 1987). It nest on small pools or lakes, showing a preference for those in treeless areas that have well-vegetated margins and low islets or promontories on which to nest (Snow and Perrins, 1998). Outside of the breeding season this species frequents inshore waters along sheltered coasts, occurring inland occasionally on lakes, pools, reservoirs and rivers (del Hoyo *et al.*, 1992; Snow and Perrins, 1998). This species diet comprises mostly fish as well as crustaceans, molluscs, frogs, fish spawn (del Hoyo *et al.*, 1992), aquatic insects, annelid worms (Snow and Perrins, 1998) and plant matter (del Hoyo *et al.*, 1992).

According to the IUCN Red List of Threatened Species (BirdLifeInternational, 2018), this species is classified as Least Concern (LC).

1.6.8. *Ixobrycus minutus* (Common Little Bittern; Linnaeus, 1766)

The species *Ixobrycus minutus* belongs to the Order Ciconiiformes, Family Ardeidae (BirdLifeInternational, 2018).

The common little bittern is a widespread species, occurring across Europe, western Asia, Africa, Madagascar, Australia and New Guinea (del Hoyo *et al.*, 1992; Kushlan and Hancock, 2005). This species is mainly found in freshwater wetlands with dense aquatic vegetation, preferably with deciduous trees and bushes (del Hoyo *et al.*, 1992). It may also occupy the edge of lakes, pools and reservoirs (del Hoyo *et al.*, 1992), wooded and marshy edges of streams and rivers, saltmarshes (Kushlan and Hancock, 2005), wooded swamps, wet grasslands, mangroves and margins of saline lagoons (del Hoyo *et al.*, 1992).

Its diet varies with the season and location, but normally comprises insects, such as crickets, grasshoppers, caterpillars, beetles, aquatic insects and larvae (del Hoyo *et al.*, 1992; Kushlan and Hancock, 2005). However, this species can also feed on spiders, molluscs, crustaceans (i.e., shrimp and crayfish; del Hoyo *et al.*, 1992; Kushlan and Hancock, 2005) and small vertebrates, such as fish, frogs, tadpoles, small reptiles and birds (del Hoyo *et al.*, 1992).

According to the IUCN Red List of Threatened Species (BirdLifeInternational, 2018), this species is classified as Least Concern (LC).

1.6.9. *Larus argentatus* (European Herring Gull; Pontoppidan; 1763)

The European herring gull (*Larus argentatus*) belongs to the Order Charadriiformes, Family Laridae (BirdLifeInternational, 2018).

This species inhabits coastal and near-coastal areas, but might also forage inland on large lakes and reservoirs, field and refuse dumps (del Hoyo *et al.*, 1996). It breeds preferentially on rocky sores with cliffs, outlying stacks or islets (del Hoyo *et al.*, 1996; Snow and Perrins, 1998).

It has a highly opportunistic diet, exploiting almost any superabundant source of food (del Hoyo *et al.*, 1996). It feeds on fish, earthworms, crabs and other marine invertebrates (i.e., molluscs, starfish or marine worms), adult and young birds, bird eggs, rodents, insects (i.e., ants), berries and tubers (i.e., turnips; del Hoyo *et al.*, 1996). The European herring gull also scavenges at refuse dumps, fishing wharves and sewage outfall zones and often follows fishing boats (del Hoyo *et al.*, 1996; Hüppop and Wurm, 2000).

According to the IUCN Red List of Threatened Species (BirdLifeInternational, 2018), this species is classified as Least Concern (LC).

1.6.10. *Larus audouinii* (Audouin's Gull; Payraudeau, 1826)

The species *Larus audouinii* belongs to the Order Charadriiformes, Family Laridae (BirdLifeInternational, 2018).

Audoin's gull nests mostly in the Mediterranean, along the coast of several countries, with most of the breeding population concentrated in Spain (del Hoyo *et al.*, 1996). It is a partially migratory species, wintering on the coasts of the Mediterranean and north-west Africa, to Senegal (del Hoyo *et al.*, 1996). Just over a decade ago, some individuals originating in Spanish colonies of the western Mediterranean began to nest in the eastern Algarve (Leal and Lecoq, 2006). In the Algarve, this species occurs from March to October, and winters in small numbers since the 2000s (Leal and Lecoq, 2006).

It is a coastal species, hardly occurring inland (Cramp and Simmons, 1983) and generally associated to coastal and continental shelf waters (Meirinho *et al.*, 2014). Usually forages at night, and their diet consists mostly of fish such as sardines and anchovies, being strongly related to fishing activities (Mañosa *et al.*, 2004). This species nests colonially in rocky or sandy islands, on sandy peninsulas and salt pans (Meirinho *et al.*, 2014).

According to the IUCN Red List of Threatened Species (BirdLifeInternational, 2018), this species is classified as Least Concern (LC).

1.6.11. *Larus fuscus* (Lesser Black-backed Gull; Linnaeus, 1758)

Larus fuscus belongs to the Order Charadriiformes, Family Laridae (BirdLifeInternational, 2018).

Specimens breed from central-north of Russia, around Scandinavia, Germany, Belgium, Nederland and northern United Kingdom to Iceland. Moreover, it breeds all year-round on the coast of Portugal, South of Ireland, United Kingdom and North of France, and one seasonally breeding population can be found in the north-east of Spain (del Hoyo *et al.*, 1996).

This species can be found in a variety of coastal habitats, including estuaries, harbours and lagoons, as well as in inland artificial habitats, such as rubbish dumps and agricultural fields (del Hoyo *et al.*, 1996). Most populations of this species are entirely migratory (del Hoyo *et al.*, 1996).

This species is omnivorous, opportunistic feeder (BirdLifeInternational, 2000) that forages extensively at sea (BirdLifeInternational, 2018). The diet consists of fish, discarded bycatch (marine and aquatic crustaceans and bivalves) and debris from landfills and sewage exists, among others (Catry *et al.*, 2010).

According to the IUCN Red List of Threatened Species (BirdLifeInternational, 2018), this species is classified as Least Concern (LC).

1.6.12. *Larus melanocephalus* (Mediterranean Gull; Temmink, 1820)

The species *Larus melanicephalus* belongs to Order Charadriiformes, Family Laridae (BirdLifeInternational, 2018).

The Mediterranean gull has a distribution that is essentially confined to Europe, nesting from Russia and the Ukrainian coast of the Black Sea to southern France and Spain, with nesting populations located throughout central Europe and the Mediterranean (del Hoyo *et al.*, 1996). This species winters along the coasts of the Black Sea, Mediterranean, European Atlantic and north-east Africa (del Hoyo *et al.*, 1996).

Most of this species populations are fully migratory and travel along coastlines between their breeding and wintering areas (del Hoyo *et al.*, 1996; Olsen, 2010). During breeding season this species diet consists of terrestrials and aquatic insects, gastropods, fish and rodents (del Hoyo *et al.*, 1996). Their diet in the non-breeding season includes marine fish, molluscs (del Hoyo *et al.*, 1996; Urban *et al.*, 1986), insects (i.e., beetles and grasshoppers; del Hoyo *et al.*, 1996; Milchev *et al.*, 2004), earthworms, berries (Urban *et al.*, 1986), seeds (i.e., barley, wheat, sunflowers and ragwort; Milchev *et al.*, 2004) offal and occasionally sewage and waste (del Hoyo *et al.*, 1996).

According to the IUCN Red List of Threatened Species (BirdLifeInternational, 2018), this species is classified as Least Concern (LC).

1.6.13. *Larus michahellis* (Yellow-legged Gull; J. F. Naumann, 1840)

Larus michahellis belongs to the Order Charadriiformes, Family Laridae (BirdLifeInternational, 2018).

This species can be found in Europe, Middle East and north Africa (del Hoyo *et al.*, 1996). Is resident in much of southern Europe, Mediterranean coast, Black and Caspian Sea, Azores and Madeira, Continental Portugal, Canary Islands and Spain (del Hoyo *et al.*, 1996). They winter on the south-west coast of Asia, most of the European coast up to Denmark and the coast of Africa from western Sahara trough the eastern Mediterranean (del Hoyo *et al.*, 1996).

During breeding season, this gull species can be found in different habitats, but calm places are preferred by this species, such as small islands or coastal cliffs, to nest (Guedes and Costa, 1994). However, it is increasingly colonizing areas associated to human activities due to the destruction of their natural habitats (Guedes and Costa, 1994). Populations can be either dispersive or sedentary (del Hoyo *et al.*, 1996). The diet consists of fish, invertebrates (i.e., insects and molluscs; Olsen, 2010), reptiles, small mammals, bird eggs and chicks (del Hoyo *et al.*, 1996).

According to the IUCN Red List of Threatened Species (BirdLifeInternational, 2018), this species is classified as Least Concern (LC).

1.6.14. *Larus ridibundus* (Black-headed Gull; Linnaeus, 1766)

Specimens of *Larus ridibundus* belongs to the Order Charadriiformes, Family Laridae (BirdLifeInternational, 2018).

This bird nests on the European continent, southern Greenland, in central Asia to the extreme south-east of Russia and some in North America (Meirinho *et al.*, 2014). It winters the most in European regions south of the nesting areas, on the African coast of the north hemisphere and the temperate and tropical coasts (north of the equator) of the Asian continent (del Hoyo *et al.*, 1996). In Continental Portugal, the majority of the population occurs during fall and winter (Meirinho *et al.*, 2014). However, black-headed gulls can be observed all year round due to the presence of non-breeding individuals (Catry *et al.*, 2010; Leitão *et al.*, 1997).

The black-headed gull inhabits coastal areas, preferring estuarine and lagoon areas, saltmarshes, aquacultures and beaches near the river mouth (Catry *et al.*, 2010). They also occur near dams, pastures, agricultural land, landfills and wastewaters treatment plants (Catry *et al.*, 2010; Elias *et al.*, 1998). This is an opportunistic species that feeds essentially on aquatic (mainly mollusks) and terrestrial (mainly worms and insects) invertebrates and sometimes fish (Catry *et al.*, 2010; del Hoyo *et al.*, 1996).

According to the IUCN Red List of Threatened Species (BirdLifeInternational, 2018), this species is classified as Least Concern (LC).

1.6.15. *Melanita nigra* (Common Scoter; Linnaeus, 1758)

The common scoter (*Melanitta nigra*) belongs to the Order Anseriformes, Family Anatidae (BirdLifeInternational, 2018).

This species breeds across northern Europe and northern Russia, including Iceland, Greenland, Scandinavia and the northern United Kingdom (BirdLifeInternational, 2018; Kear, 2005; Madge and Burn, 2010). Specimens winter along inshore coastal waters of western Europe and western North Africa, from Norway south to Mauretania and in the Baltic Sea (del Hoyo *et al.*, 1992; Kear, 2005; Madge and Burn, 2010).

This species breeds around freshwater lakes, pools, rivers and streams in tundra and in open habitats in sub-Arctic areas (del Hoyo *et al.*, 1992; Kear, 2005; Madge and Burn, 2010). Areas with suitable nesting cover are preferred by individuals of this species (del Hoyo *et al.*, 1992; Johnsgard, 1978; Kear, 2005), but wetlands that are enclosed by forest tend to be avoid (Kear, 2005). Although they may be found inland, freshwater lakes during its migration, the common scoter overwinters at sea, where it can be found in shallow, inshore waters, bays and estuary mouths (Kear, 2005; Madge and Burn, 2010).

The diet of this species consists mainly of molluscs, predominantly mussels (del Hoyo *et al.*, 1992), although it may also feed other aquatic invertebrates, such as crustaceans (i.e., barnacles and shrimps; del Hoyo *et al.*, 1992; Johnsgard, 1978), worms (del Hoyo *et al.*, 1992), echinoderms, isopods, amphipods (Kear, 2005) and insects (i.e., midges and caddisflies), as well as small fish (del Hoyo *et al.*, 1992) and fish eggs (Snow and Perrins, 1998). However, during breeding season they may also ingest plant matter (del Hoyo *et al.*, 1992).

According to the IUCN Red List of Threatened Species (BirdLifeInternational, 2018), this species is classified as Least Concern (LC).

1.6.16. *Morus bassanus* (Northern Gannet; Linnaeus, 1758)

Specimens of *Morus bassanus* belongs to the Order Pelecaniformes, Family Sulidae (BirdLifeInternational, 2018).

This species can be found in both sides of the Atlantic Ocean with small numbers of individuals reaching the equator and Norway (Gooders *et al.*, 1996). Breeding sites includes the northern France, the United Kingdom, Ireland, Norway and western Quebec (Canada; del Hoyo *et al.*, 1992). The northern gannet winters in the Atlantic Ocean and Mediterranean Sea (Blomdahl *et al.*, 2003).

It is a strictly marine species that forages mainly over continental shelves, feeding on shoaling pelagic fish that are mostly caught by plunge-diving from large heights (BirdLifeInternational, 2018).

According to the IUCN Red List of Threatened Species (BirdLifeInternational, 2018), this species is classified as Least Concern (LC).

1.6.17. *Phalacrocorax carbo* (Great Cormorant; Linnaeus, 1758)

The species *Phalacrocorax carbo* belongs to the Order Pelecaniformes, Family Phalacrocoracidae (BirdLifeInternational, 2018).

The great cormorant has an extremely large distribution and can be found on every continent, with exception of South America and Antarctica (Paterson, 1997). In Europe, it can be found along the Atlantic coast, Mediterranean and in large areas of Eastern Europe (Gooders *et al.*, 1996). Breeding colonies are found in western Greenland to Denmark (Gooders *et al.*, 1996).

In marine environments, this species can occur in sheltered coastal areas on estuaries, saltpans, lagoons and coastal bays, but they can also occur in terrestrial environments, such as lakes, reservoirs, wide rivers and swamps (Peterson *et al.*, 1987). This species feeds mostly on fish that captures through small or medium depth diving's (del Hoyo *et al.*, 1992). In Portugal, several studies revealed a generalist diet that, in estuarine and coastal lagoon environments, may consist of fish such as mullets, toadfish, eel and several species of cardinalfish and sole (Catry *et al.*, 2010; Dias *et al.*, 2012; Granadeiro *et al.*, 2013).

According to the IUCN Red List of Threatened Species (BirdLifeInternational, 2018), this species is classified as Least Concern (LC).

1.6.18. *Platalea leucorodia* (Eurasian Spoonbill; Linnaeus, 1758)

The Eurasian spoonbill (*Platalea leucorodia*) belongs to the Order Ciconiiformes, Family Threskiornithidae (BirdLifeInternational, 2018).

This species has a wide but fragmented Palearctic distribution, breeding from Europe to Northwest Africa, Red Sea, India and China (Cramp and Simmons, 1983; Hancock *et al.*, 1992). It winters in the Atlantic coast of Europe, Mediterranean, sub-Saharan countries, Pakistan, Iran, India, Sri Lanka and southern China (Triplet *et al.*, 2008).

The Eurasian spoonbill inhabits fresh, brackish or saltmarshes (Hancock *et al.*, 1992; Snow and Perrins, 1998), estuaries, deltas, tidal creeks, rivers, lakes, reservoirs and mangrove swamps(del Hoyo *et al.*, 1992; Hancock *et al.*, 1992; Triplet *et al.*, 2008), showing a preference for shallow wetlands with mud, clay or fine sand bottom (del Hoyo *et al.*, 1992).

It forages alone or in small groups, wading methodically through shallow waters as sweeping its bill from side to side in search for prey (del Hoyo *et al.*, 1992). Molluscs, crustaceans, worms, leeches, frogs, tadpoles, adult and larval insects (i.e., water beetles, dragonflies, caddisflies, locusts and flies) and small fish comprises this species diet, however it may also take algae and small fragments of aquatic plants (del Hoyo *et al.*, 1992), although these might be ingested accidentally (Hancock *et al.*, 1992).

According to the IUCN Red List of Threatened Species (BirdLifeInternational, 2018), this species is classified as Least Concern (LC).

1.6.19. *Rissa tridactyla* (Black-legged Kittiwake; Linnaeus, 1758)

Rissa tridactyla belongs to the Order Charadriiformes, Family Laridae (BirdLifeInternational, 2018).

The black-legged kittiwake nests on coastlines and islands across the North Pacific and North Atlantic Oceans, as on islands off the northern coasts of Russia and Norway (del Hoyo *et al.*, 1996; Hatch *et al.*, 2009). This species breeds in the North Atlantic, from northern Canada and northern United States, through Greenland, northern and western Europe and on the Taymyr Peninsula and Severnaya Zemlya in Russia (del Hoyo *et al.*, 1996; Hatch *et al.*, 2009).

It is a migratory species that disperses after the breeding season from coastal areas to the open ocean (del Hoyo *et al.*, 1996). This species nests on high, steep coastal cliffs with narrow ledges in areas with easy access to freshwater (del Hoyo *et al.*, 1996). It is highly pelagic during winter, remaining on the wing out of sight of land (del Hoyo *et al.*, 1996). Black-legged kittiwake's diet consists mainly of marine invertebrates (i.e., squid and shrimps) and fish, although during the breeding season it might also feed on intertidal molluscs, crustaceans (i.e., crayfish; del Hoyo *et al.*, 1996; Flint *et al.*, 1984), earthworms, small mammals and plant matter (i.e., aquatic plants, potato tubers and grain; del Hoyo *et al.*, 1996). While at sea during winter it will also feed on planktonic invertebrates and regularly exploit sewage outfalls and fishing vessels (del Hoyo *et al.*, 1996).

According to the IUCN Red List of Threatened Species (BirdLifeInternational, 2018), this species is classified as Vulnerable (VU).

2. Materials and Methods

2.1.Sampling

Birds' samples were collected from a total of 348 individuals (Table 2.1) at five different wildlife rescue centres (WRC) from North to South Portugal, Parque Biológico de Gaia (PBGaia), Centro de Ecologia, Recuperação e Vigilância de Animais Selvagens (CERVAS), Centro de Estudos e Recuperação de Animais Selvagens (CERAS), Centro de Recuperação de Animais Silvestres de Lisboa (LxCRAS) and Centro de Recuperação e Investigação de Animais Selvagens (RIAS; Figure 2.1).

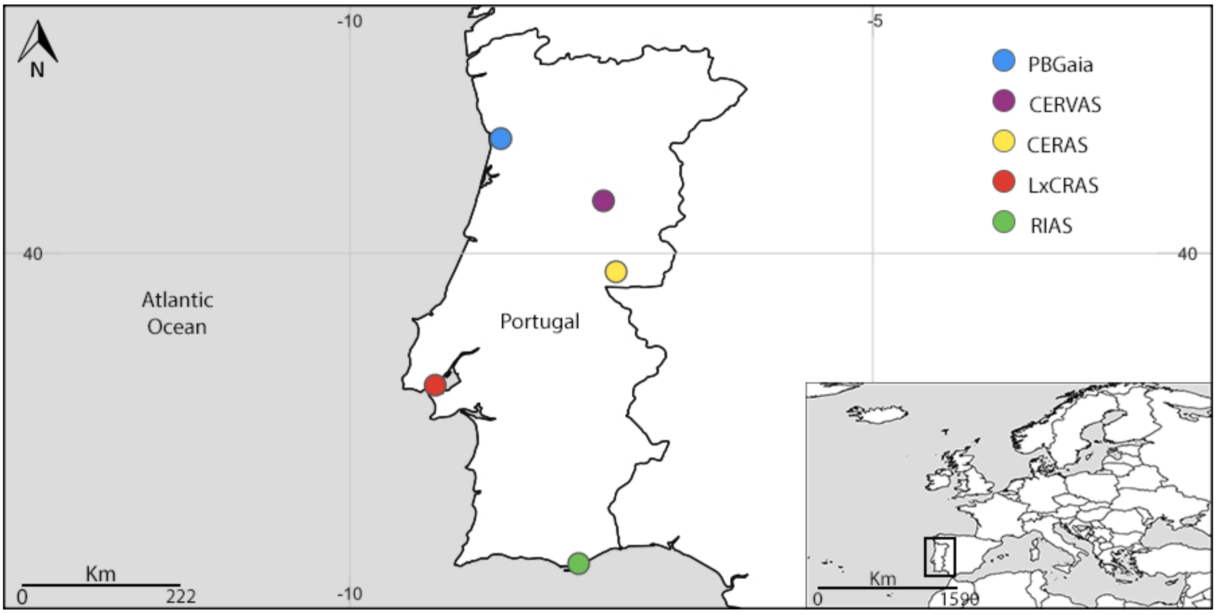

Figure 2.1: Points represent the five wildlife rescue centres that collaborated in this study.

As a result of injury, illness and exhaustion, birds were found stranded in various locations along the Portuguese coast and brought into care at the rescue centres either dead or died during their stay. Each bird was properly labelled, weighted on an electronic balance to the nearest g and kept frozen at -20°C until dissections were performed.

In this study, three datasets were assembled. Dataset A includes samples obtained from all the WRC and was used for a baseline assessment of the prevalence of plastic litter affecting multispecies populations of aquatic-associated birds in Portugal. Dataset B only included samples collected by the southern rescue centre, RIAS, and was used to (1) test the effect of age and gender on plastic ingestion and to (2) assess the correlation between the physical condition of the birds and the plastic debris ingested. Dataset C only included White Stork (*Ciconia ciconia*) samples collected by all WRC and was used to (1) test for differences in the type, colour and polymer of plastics ingested by northern and southern samples and (2) determine whether plastic ingestion increased in last seven years. *Ciconia ciconia* samples from all rescue centres were divided into northern or southern regions, based on location of collection by volunteers and consequently the geographic position of the rescue centre involved. Specifically, the northern region consists of *C. ciconia* samples collected by CERVAS and CERAS, while the southern region comprises of *C. ciconia* samples from LxCRAS and RIAS.

2.2.Dissections

Before dissections, birds were thawed at room temperature. Dissections were performed following the standard dissection methodology of Van Franeker (2004). The application of this methodology structured the recording of a wide range of data needed to assess origin, body condition, probable cause of death, age, gender and other potentially relevant issues (Van Franeker *et al.*, 2011; see Suplemental Material - Table S1). Briefly, carcasses were dissected along the anteroposterior axis between the breastbone and cloaca (Figure 2.2). Body condition was recorded considering the condition of the pectoral muscle and was assessed by its palpation using a scale of 1 (lean) to 5 (obese; Carrega, 2016). This condition is an important guide to the overall nutritional state of the bird (Krautwald-Junghanns *et al.*, 2008), since it may be correlated to the cause of death and/or duration of the process of dying, which may also be linked to the stomach contents, including litter (Van Franeker, 2004). As body condition deteriorates, birds usually deplete their fat reserves first and then start using proteins from the muscles, such as the pectoral muscle (Van Franeker, 2004). Gender and age were determined considering the development stage of the sexual organs and plumage evaluation, respectively. The oesophagus and stomach collected were properly preserved in aluminium foil, labelled and kept frozen at -20°C until further analyses.

Table 2.1: Sample description. Male (M) or female (F), chick (C), juvenile (J), sub-adult (S-A) or adult (A). Note that gender and/or age could not always be determined.

Wildlife Rescue Centres	Species	Sample size (n)	Gender		Age			
			M	F	C	J	S-A	A
PBGaia	*Larus michahellis*	20	7	11	0	8	1	11
	Morus bassanus	2	2	0	0	0	1	1
	Ardea cinerea	1	1	0	-	-	-	-
	Larus argentatus	1	1	0	0	0	1	0
	Larus fuscus	1	0	1	0	0	0	1
CERVAS	*Ciconia ciconia*	33	11	12	9	9	0	15
	Ardea cinerea	13	6	3	0	7	0	4
	Larus fuscus	2	0	1	0	2	0	0
	Melanitta nigra	2	-	-	0	2	0	0
	Alca torda	1	-	-	-	-	-	-
	Ciconia nigra	1	-	-	0	1	0	0
	Gavia stellata	1	-	-	0	1	0	0
	Larus michahellis	1	-	-	0	1	0	0
	Platalea leucorodia	1	1	0	0	1	0	0
	Rissa tridactyla	1	0	1	0	0	0	1
CERAS	*Ciconia ciconia*	14	6	1	3	4	0	7
	Phalacrocorax carbo	1	-	-	0	0	0	1
LxCRAS	*Larus fuscus*	36	9	6	-	-	-	-
	Larus michahellis	7	1	4	-	-	-	-
	Ciconia ciconia	1	1	0	-	-	-	-
	Morus bassanus	1	1	0	-	-	-	-
	Larus ridibundus	1	0	1	-	-	-	-
RIAS	*Larus michahellis*	96	21	26	3	33	8	35
	Larus fuscus	68	22	14	0	20	7	33
	Morus bassanus	18	6	0	0	9	3	3
	Ciconia ciconia	10	4	4	0	2	0	7
	Bubulcus ibis	4	1	1	0	0	0	1
	Ardea cinerea	3	1	1	0	0	0	3
	Larus ridibundus	3	0	1	0	0	2	1
	Egretta garzetta	1	-	-	-	-	-	-
	Ixobrychus minutus	1	-	-	-	-	-	-
	Larus audouinii	1	-	-	0	1	0	0
	Larus melanocephalus	1	0	1	0	0	1	0

Figure 2.2: Initial skin incision in the dissection, from over the breastbone to near the cloaca. After this incision, body condition was scored based on the condition of the pectoral muscle, which is assessed by its palpation.

After being thawed at room temperature, the stomachs were weighted using an electronic balance (Sartorius Advantage AW-224 Balance) to the nearest 0.0001 g. Then, they were carefully opened and examined for perforations, lacerations, ulceration or hemorrhage. Stomach contents were examined for the presence of plastics or other foreign matter. The contents were carefully rinsed in a metal sieve with a 1 mm mesh and the remain items were transferred to a glass petri dish left to dry in the oven at 40°C overnight. A 1 mm mesh was used, because smaller meshes become easily clogged with mucus from the stomach wall and food remains (Van Franeker *et al.*, 2011).

Plastic items were counted and classified according to Van Franeker *et al.* (2011) into industrial- or user-plastics, with the later further subdivided into sheetlike (e.g., plastic bags), threadlike (e.g., fishing line and rope), foamed, fragments and others (e.g., rubber). They were also counted and sorted based on Kain *et al.* (2016) into the following colour categories: white (including clear), black (including grey and brown), yellow, green, red (including pink), blue and mixed. Maximum length (± 1 mm) of each plastic item was recorded using a grid paper, being afterwards counted and sorted into the size categories proposed by Barnes *et al.* (2009) since is the most relevant and applicable classification as includes extra-large sizes of plastics that are usually ingested by marine megafauna (i.e., marine mammals, turtles and seabirds; Provencher *et al.*, 2017). This classification includes megaplastics (> 100 mm), macroplastics (> 20 – 100 mm), mesoplastics (> 5 – 20 mm) and microplastics (1 - 5 mm; Barnes *et al.*, 2009). For each stomach, total plastic weight and weight by plastic categories was measured using an electronic balance (Sartorius Advantage AW-224 Balance) to nearest 0.0001 g.

To characterize polymer composition, a representative subsample of all plastic categories of dataset C was analysed using Micro Raman (μ-Raman) spectroscopy (JASCO NRS-4100). A 5x or 20x objective was used to focus a laser beam (532 or 785 mm) on the sample surface, which resulted in a spot size of ~30 or ~5 μm, respectively; considering the specific sample, the laser power was in the 0.5 – 5.0 mW range but was kept low enough to prevent sample damage. Since the μ-Raman spectrometer has a high spatial resolution, at least three spectra at three different points of each sample surface were acquired. To identify polymer composition the spectra obtained were then compared with the spectra of the most common polymers included in a home-made spectral database. Polymer identification through μ-Raman can be sometimes ambiguous or not possible, mostly due to intense photoluminescence background, so when not possible, Fourier-Transform Infra-Red (FT-IR) spectroscopy was used as an additional technique (JASCO FT/IR-4700), performing both transmission and attenuated total reflectance (ATR) measurements.

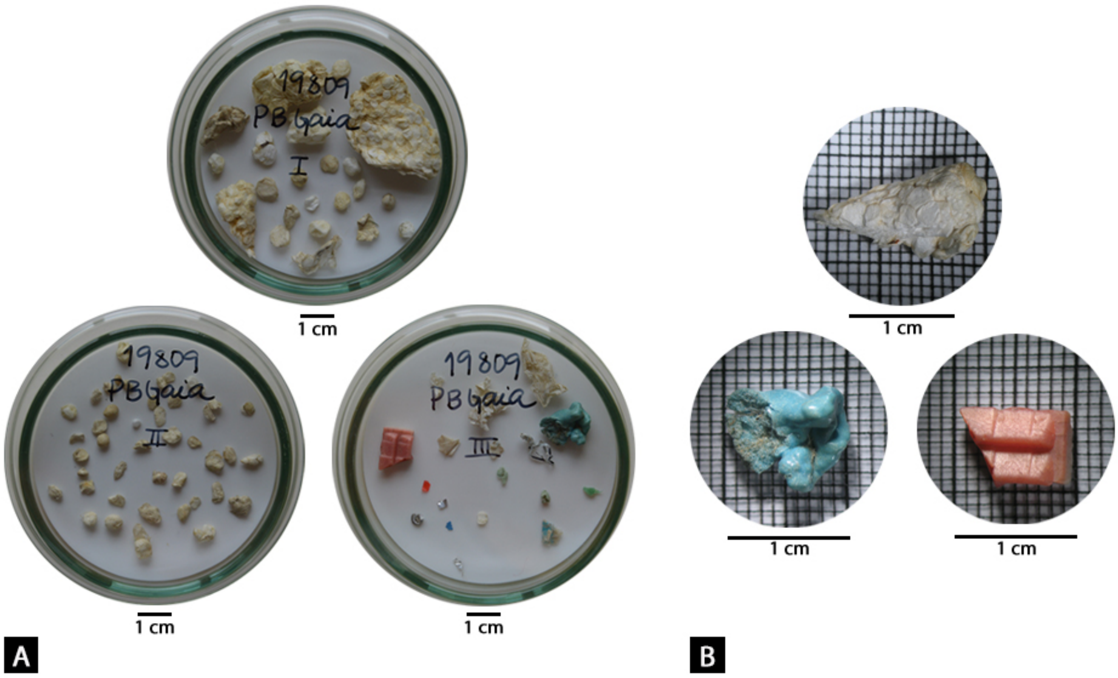

Figure 2.3: (A) Example of a stomach content of a *Larus michahellis*. All subcategories of user plastics (sheetlike, threadlike, foamed, fragment and others) were retrieved in this sample. (B) Example of how the items were sorted, photographed and measured to the maximum length (± 1 mm) using a grid paper.

2.3.Statistical analysis

For each species, the percentage frequency of occurrence (% FO) and abundance (i.e., average number and mass of pieces of plastics using all individuals examined) of plastics was recorded (Provencher *et al.*, 2017).

To test if the incidence of plastics differed among species, dataset A was used. To do so, two separate permutational univariate analyses (PERMANOVA) were performed on either the number or total mass of plastics with species as the independent factor.

To test if gender or age groups had an effect on (1) incidence of plastics (measured as number or total mass) and (2) type and colour of plastic ingested, dataset B was used. For (1), the entire dataset was used and four separate univariate permutational analyses (PERMANOVA) were performed on either number or total mass of plastics with gender or age group as independent factor. For (2), only affected birds were used and four separate multivariate permutational analyses (PERMANOVA) were performed on the abundance of plastic type (i.e., dependent variables: industrial, sheetlike, threadlike, foamed, fragments and other) or plastic colour (i.e., dependent variables: white, black, yellow, green, red, blue and mixed) with gender or age group as the independent factor.

To test if the origin of *C. ciconia* samples (i.e., northern or southern Portugal) had an effect on (1) incidence of plastics (measured as number or total mass) and (2) type, colour or polymer of plastic debris, dataset C was used. For (1), the entire dataset was used and two separate univariate permutational analyses (PERMANOVA) were performed on either number or total mass of plastics with region as independent factor. For (2), only affected birds were used and three separate multivariate permutational analyses (PERMANOVA) were performed on the abundance of either plastic type (i.e., dependent variables: industrial, sheetlike, threadlike, foamed, fragments and other) or plastic colour (i.e., dependent variables: white, black, yellow, green, red, blue and mixed) or plastic polymer (i.e., dependent variables: polydimethylsiloxane, polyamide, polystyrene, polyethylene and polypropylene) with region as the independent factor.

In all tests, post-hoc comparisons were performed using pair-wise tests while Monte Carlo P-value was preferred over the permutational P-value when very few unique permutations were possible (Anderson, 2005). For each multivariate analysis, a Bray-Curtis distance dissimilarity matrix was used for square root transformed multivariate measures. Permutation tests of multivariate dispersion (PERMDISP; Anderson, 2004) were used to check the homogeneity in the average dissimilarities of samples from the central location of their group. Similarity percentage procedure SIMPER (Clarke, 1993) was used to assess the percentage of contribution (%) that each dependent variable had in the Bray-Curtis dissimilarities with a cut off point for low contributions set at 90%. Principal Coordinate Analysis (PCO) were used to visualize the multivariate data.

To test whether there is a statistical significant relationship between body condition and number or total mass of plastic items, correlations were run on the specimens that showed plastic contents from dataset B.

To evaluate whether plastic ingestion changed over time, data of specimens that showed plastic contents from dataset C was used and time trends of number or total mass of plastic items were evaluated by Simple Linear Regressions.

For statistical purpose, only representative species were used, as the remaining species showed a low number of birds. Prior to each analysis, all data was standardized to the weight of the respective stomach. All tests were performed with the software package PRIMER 6 v6.1.13 & PERMANOVA+ v1.0.3 (Clarke and Gorley, 2006) with the exception of the correlations and regressions where STATISTICA v13.2 (DellInc., 2016) was used instead.

3. Results

3.1. Dataset A

Dataset A is composed by 348 birds from nineteen species (Supplemental material – Table S2). Analyses were restricted to the following four species that had a similar sample size: (1) White Stork (*Ciconia ciconia*, CC), (2) Lesser Black-backed Gull (*Larus fuscus*, LF), (3) Yellow-legged Gull (*Larus michahellis*, LM) and (4) Northern Gannet (*Morus bassanus*, MB). Of the 310 birds analysed, 49 individuals (frequency of occurrence of 15.48%) ingested plastics, on average number of 1.62 (± 10.19 SD) items per individual, on average 0.0771 g (± 0.56 SD) and on average a plastic item of 0.75 mm in length (± 7.36 SD).

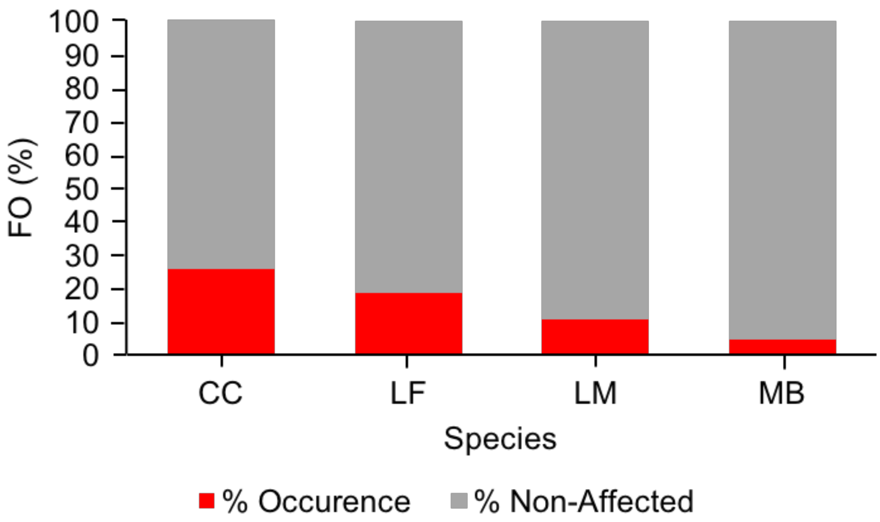

Figure 3.1: Percentage frequency of occurrence (% FO) of plastic litter in the stomach of four aquatic birds' species.

Ciconia ciconia was the species with the highest frequency of plastics. Among the 58 *C. ciconia* stomachs examined, 25.86% had plastic debris (Figure 3.1; Table 3.1). *Larus fuscus* accumulated on average more plastic items than the other species (1.85 ± 9.63; ± 0.93) but lighter in weight compared to *C. ciconia* (Table 3.1 and 3.2).

Table 3.1: Data on the plastics ingested by *Ciconia ciconia* (n = 58) based on plastics categories. Frequency of occurrence of plastics (with Jeffery's nominal 95% confidence intervals – CI) and plastic litter abundance. Abundance was calculated including all individuals sampled (affected and non-affected).

	Frequency of occurrence (%; 95% CI)	Number of plastic items			Mass of plastic items		
		Mean (n; ± sd; ± se)	Median	Range	Mean (g; ± sd; ± se)	Median	Range
Global	25.86 (0.16 – 0.38)	1.41 (± 4.97; ± 0.65)	0	0 – 35	0.2441 (± 1.09; ± 0.14)	0	0 – 7.6339
Industrial	0 (0 – 0.04)	0	0	0	0	0	0
User	25.86 (0.16 – 0.38)	1.41 (± 4.97; ± 0.65)	0	0 – 35	0.2441 (± 1.09; 0.14)	0	0 – 7.6339
Sheetlike	12.07 (0.06 – 0.22)	0.29 (± 0.99; ± 0.13)	0	0 – 5	0.0087 (± 0.05; ± 0.01)	0	0 – 0.3551
Threadlike	3.45 (0.01 – 0.11)	0.14 (± 0.93; ± 0.12)	0	0 – 7	0.0183 (± 0.13; ± 0.02)	0	0 – 1.0145
Foam	3.45 (0.01 – 0.11)	0.03 (± 0.18; ± 0.02)	0	0 – 1	0.0009 (± 0.01; ± 0.001)	0	0 – 0.0483
Fragments	12.07 (0.06 – 0.22)	0.24 (± 0.88; ± 0.12)	0	0 – 6	0.0035 (± 0.01; ± 0.002)	0	0 – 0.0552
Other	10.34 (0.04 – 0.20)	0.71 (± 3.32; ± 0.44)	0	0 – 24	0.1567 (± 1.01; ± 0.13)	0	0 – 7.6339

Table 3.2: Data on the plastics ingested by *Larus fuscus* (n = 107) based on plastics categories. Frequency of occurrence of plastics (with Jeffery's nominal 95% confidence intervals – CI) and plastic litter abundance. Abundance was calculated including all individuals sampled (affected and non-affected).

	Frequency of occurrence (%; 95% CI)	Number of plastic items			Mass of plastic items		
		Mean (n; ± sd; ± se)	Median	Range	Mean (g; ± sd; ± se)	Median	Range
Global	18.69 (0.12 – 0.27)	1.85 (± 9.63; ± 0.93)	0	0 – 91	0.0781 (± 0.48; ± 0.05)	0	0 – 4.0969
Industrial	2.80 (0.01 – 0.07)	0.87 (± 8.70; ± 0.84)	0	0 – 90	0.0311 (± 0.32; ± 0.03)	0	0 – 3.2657
User	16.82 (0.11 – 0.25)	0.98 (± 4.14; ± 0.40)	0	0 – 32	0.0071 (± 0.12; ± 0.01)	0	0 – 2.7455
Sheetlike	7.48 (0.04 – 0.14)	0.51 (± 2.71; ± 0.26)	0	0 – 20	0.0283 (± 0.27; ± 0.03)	0	0 – 2.7455
Threadlike	2.80 (0.01 – 0.07)	0.06 (± 0.36; ± 0.03)	0	0 – 3	0.0001 (± 0.0004; ± 0.00004)	0	0 – 0.0030
Foam	1.87 (0.004 – 0.06)	0.11 (± 1.07; ± 0.10)	0	0 – 11	0.0001 (± 0.001; ± 0.0001)	0	0 – 0.0094
Fragments	4.67 (0.02 – 0.10)	0.09 (± 0.54; ± 0.05)	0	0 – 5	0.0009 (± 0.01; ± 0.001)	0	0 – 0.0610
Other	6.54 (0.03 – 0.12)	0.21 (± 1.47; ± 0.14)	0	0 – 15	0.0059 (± 0.04; ± 0.004)	0	0 – 0.3252

Table 3.3: Data on the plastics ingested by *Larus michahellis* (n = 124) based on plastics categories. Frequency of occurrence of plastics (with Jeffery's nominal 95% confidence intervals – CI) and plastic litter abundance. Abundance was calculated including all individuals sampled (affected and non-affected).

	Frequency of occurrence (%; 95% CI)	Number of plastic items			Mass of plastic items		
		Mean (n; ± sd; ± se)	Median	Range	Mean (g; ± sd; ± se)	Median	Range
Global	10.48 (0.06 – 0.17)	0.80 (± 7.11; ± 0.64)	0	0 – 79	0.0053 (± 0.04; ± 0.004)	0	0 – 0.4614
Industrial	0 (0 – 0.02)	0	0	0	0	0	0
User	10.48 (0.06 – 0.17)	0.80 (± 7.11; ± 0.64)	0	0 – 79	0.0010 (± 0.01; ± 0.001)	0	0 – 0.2861
Sheetlike	4.84 (0.02 – 0.10)	0.13 (± 1.00; ± 0.09)	0	0 – 11	0.0004 (± 0.003; ± 0.0003)	0	0 – 0.0317
Threadlike	2.42 (0.01 – 0.06)	0.04 (± 0.30; ± 0.03)	0	0 – 3	0.00003 (± 0.0002; ± 0.00004)	0	0 – 0.0016
Foam	1.61 (0.003 – 0.05)	0.53 (± 5.84; ± 0.52)	0	0 – 65	0.0023 (± 0.03; ± 0.002)	0	0 – 0.2861
Fragments	4.03 (0.02 – 0.09)	0.08 (± 0.56; ± 0.05)	0	0 – 6	0.0017 (± 0.01; ± 0.001)	0	0 – 0.1300
Other	1.61 (0.003 – 0.05)	0.02 (± 0.13; ± 0.01)	0	0 – 1	0.0008 (± 0.01; ± 0.001)	0	0 – 0.0766

Table 3.4: Data on the plastics ingested by *Morus bassanus* (n = 21) based on plastics categories. Frequency of occurrence of plastics (with Jeffery's nominal 95% confidence intervals – CI) and plastic litter abundance. Abundance was calculated including all individuals sampled (affected and non-affected).

	Frequency of occurrence (%; 95% CI)	Number of plastic items			Mass of plastic items		
		Mean (n; ± sd; ± se)	Median	Range	Mean (g; ± sd; ± se)	Median	Range
Global	4.76 (0.01 – 0.20)	5.81 (± 26.62; ± 5.81)	0	0 – 122	0.0032 (± 0.01; ± 0.003)	0	0 – 0.0676
Industrial	0 (0 – 0.11)	0	0	0	0	0	0
User	4.76 (0.01 – 0.20)	5.81 (± 26.62; ± 5.81)	0	0 – 122	0.0006 (± 0.01; ± 0.001)	0	0 – 0.0676
Sheetlike	0 (0 – 0.11)	0	0	0	0	0	0
Threadlike	0 (0 – 0.11)	0	0	0	0	0	0
Foam	4.76 (0.01 – 0.20)	5.81 (± 26.62; ± 5.81)	0	0 – 122	0.0032 (± 0.01; ± 0.003)	0	0 – 0.0676
Fragments	0 (0 – 0.11)	0	0	0	0	0	0
Other	0 (0 – 0.11)	0	0	0	0	0	0

Table 3.5: Characterization of the plastics (size and colour) found in the seven species in study.

Species	Plastic size category (%)				Plastic colour (%)						
	Microplastic (1–5 mm)	Mesoplastic (>5–20 mm)	Macroplastic (>20–100 mm)	Megaplastic (>100 mm)	White	Black	Yellow	Green	Red	Blue	Mixed
Ciconia ciconia	36.59	40.24	15.85	7.32	34.15	46.34	3.66	1.22	2.44	12.20	0
Larus fuscus	66.67	26.77	6.06	0.51	45.96	47.98	1.52	1.01	1.01	2.53	0
Larus michahellis	69.70	27.27	3.03	0	85.86	2.02	0	4.04	2.02	3.03	3.03
Morus bassanus	100	0	0	0	100	0	0	0	0	0	0

Most of the items were categorised as user plastics. Industrial plastics were only found in the species *L. fuscus* (n=3; Table 3.2). Within user plastics, items belonging to the sub-category foam were the most frequently reported followed by sheetlike, other, fragments and threadlike plastics (Table 3.1 – 3.4). Among species, different subtypes of user plastics were predominant. For example, *L. michahellis* and *M. bassanus* mainly ingested foam, while the sheetlike and other sub-categories were the most abundant in *L. fuscus* and *C. ciconia*, respectively.

Microplastic was the most common size category in all species, followed by meso-, macro-, and megaplastics (Table 3.5), indicating that smaller plastic particles are more bioavailable and have a higher chance of being accidentally or selectively ingested than larger items (Lusher *et al.*, 2015).

Overall, white coloured plastics was the most ingested followed by black, blue, green, yellow, red and mixed colours. However, interspecific differences were also observed; white coloured items were the most common in *L. michahellis* and *M. bassanus*, while black coloured plastics was the predominant type ingested by *L. fuscus* and *C. ciconia*.

There was no significant difference in the incidence of plastic debris ingested by the different species of this study (PERMANOVA, *P* (MC) = 0.244; Table 3.6) and the dispersion did not significantly differ among species (PERMDISP, *P* = 0.226). There was also no significant difference in the mass of plastic debris found among the different species (PERMANOVA, *P* (MC) = 0.103; Table 3.7) and there was no difference in dispersion among them (PERMDISP, *P* = 0.084).

Table 3.6: PERMANOVA results of the model computed to test for differences in the number of plastic debris ingested among four different species (i.e., *Ciconia ciconia*, *Larus fuscus*, *Larus michahellis* and *Morus bassanus*). Significance level was set as < 0.05.

Source	df	SS	MS	Pseudo - *F*	*P* (perm)	Unique perms	*P* (MC)
Species	3	188.26	62.752	1.3597	0.24	998	0.244
Residual	301	13892	46.153				
Total	304	14080					

Table 3.7: PERMANOVA results of the model computed to test for differences in the total mass of plastic debris ingested among four different species (i.e., *Ciconia ciconia*, *Larus fuscus*, *Larus michahellis* and *Morus bassanus*). Significance level was set as < 0.05.

Source	df	SS	MS	Pseudo - *F*	*P* (perm)	Unique perms	*P* (MC)
Species	3	18.607	6.2025	2.0692	0.086	999	0.103
Residual	301	902.25	2.9975				
Total	304	920.86					

3.2.Dataset B

Dataset B is composed by 206 birds from eleven species (Supplemental material – Table S3). Analyses were restricted to the following four species: (1) White Stork (*Ciconia ciconia*, CC), (2) Lesser Black-backed Gull (*Larus fuscus*, LF), (3) Yellow-legged Gull (*Larus michahellis*, LM) and (4) Northern Gannet (*Morus bassanus*, MB). Of the 192 birds, 12.50% (24 individuals) were affected by plastic pollution, with an average number of 1.37 (± 10.97 SD) pieces of plastic litter per bird, an average mass of 0.0321 g (± 0.30 SD) and an average length of plastic particle of 0.39 mm (± 3.44 SD).

Comparatively to Dataset A, *Ciconia ciconia* was the species that also presented the highest frequency of plastics, with 40% of the individuals affected by plastic debris (Table 3.8). *Larus fuscus* accumulated on average a similar number of plastic items but lighter in weight than those ingested in *C. ciconia* (Table 3.8 and 3.9).

Most of the items found were categorised as user plastics; items belonging to the sub-category foam was the most commonly reported, followed by other, fragments, sheetlike and threadlike plastics (Table 3.8 – 3.11). Industrial plastics were only found in the species *L. fuscus* (n=3; Table 3.9) and, in fact, it was the predominant plastic category reported in this species. Among species, different sub-categories of user plastics were predominant. For example, *C. ciconia* and *L. fuscus* mainly ingested other, while sheetlike and foam were the most abundant sub-categories in *L. michahellis* and *M. bassanus*, respectively.

Microplastic was the most common size category in all species, followed by meso-, macro- and megaplastics (Table 3.12). *Ciconia ciconia* and *L. michahellis* had high percentages of mesoplatics compared to the other species.

Overall, white coloured plastics were the most ingested followed by black, yellow, green and mixed. Interspecific differences were also observed in terms of the colour of the plastics ingested; black coloured items were the most common in *C. ciconia* and *L.*

fuscus, while white coloured plastics was the predominant type ingested by *L. michahellis* and *M. bassanus*.

The univariate analyses performed showed that there was a significant difference in the incidence of plastics among the four different age classes (i.e., chick, juvenile, sub-adult and adult; PERMANOVA, P (MC) < 0.05; Table 3.13). Pairwise tests revealed significant differences between juveniles and sub-adults and between adults and sub-adults, while all the other groups were not significantly different (Table 3.14). The variability of number of plastic debris was significantly different among age classes (PERMDISP, $P \leq 0.05$).

There was no significant difference in the total mass of plastic debris ingested among the different age classes (PERMANOVA, P (MC) = 0.079; Table 3.15) and there was a slightly significant difference in dispersion among them (PERMDISP, $P = 0.049$).

Gender had no effect on the incidence and total mass of plastic debris ingested (PERMANOVA, P (MC) = 0.851; Table 3.16; PERMANOVA, P (MC) = 0.768; Table 3.17, respectively). The variability of incidence and total mass of plastic debris did not significantly differ between genders (PERMDISP, $P = 0.902$; PERMDISP, $P = 0.758$, respectively).

The multivariate analyses performed only on affected specimens from dataset B showed that there was no significant difference in the type of plastic debris ingested among the three different age classes (i.e., juvenile, sub-adult and adult; PERMANOVA, P (MC) = 0.844; Table 3.18). The variability of plastic category did not significantly differ among age classes (PERMDISP, $P = 0.841$).

Table 3.8: Data on the plastics ingested by *Ciconia ciconia* (n = 10) based on plastics categories. Frequency of occurrence of plastics (with Jeffery's nominal 95% confidence intervals – CI) and plastic litter abundance. Abundance was calculated including all individuals sampled (affected and non-affected).

	Frequency of occurrence (%; 95% CI)	Number of plastic items			Mass of plastic items		
		Mean (n; ± sd; ± se)	Median	Range	Mean (g; ± sd; ± se)	Median	Range
Global	40.00 (0.15 – 0.70)	1.60 (± 2.46; ± 0.78)	0	0 – 6	0.1577 (± 0.26; ± 0.08)	0	0 – 0.6940
Industrial	0 (0 – 0.22)	0	0	0	0	0	0
User	40.00 (0.15 – 0.70)	1.60 (± 2.46; ± 0.78)	0	0 – 6	0.1577 (± 0.26; ± 0.08)	0	0 – 0.6940
Sheetlike	10.00 (0.01 – 0.38)	0.40 (± 1.26; ± 0.40)	0	0 – 4	0.0355 (± 0.11; ± 0.04)	0	0 – 0.3551
Threadlike	0 (0 – 0.22)	0	0	0	0	0	0
Foam	0 (0 – 0.22)	0	0	0	0	0	0
Fragments	10.00 (0.01 – 0.38)	0.20 (± 0.63; ± 0.20)	0	0 – 2	0.0027 (± 0.01; ± 0.003)	0	0 – 0.0271
Other	30.00 (0.09 – 0.61)	1.00 (± 1.94; ± 0.61)	0	0 – 6	0.1185 (± 0.24; ± 0.08)	0	0 – 0.6940

Table 3.9: Data on the plastics ingested by *Larus fuscus* (n = 68) based on plastics categories. Frequency of occurrence of plastics (with Jeffery's nominal 95% confidence intervals – CI) and plastic litter abundance. Abundance was calculated including all individuals sampled (affected and non-affected).

	Frequency of occurrence (%; 95% CI)	Number of plastic items			Mass of plastic items		
		Mean (n; ± sd; ± se)	Median	Range	Mean (g; ± sd; ± se)	Median	Range
Global	16.18 (0.09 – 0.26)	1.60 (± 11.03; ± 1.34)	0	0 – 91	0.0639 (± 0.50; ± 0.06)	0	0 – 4.0969
Industrial	4.41 (0.01 – 0.11)	1.37 (± 10.91; ± 1.32)	0	0 – 90	0.0489 (± 0.40; ± 0.05)	0	0 – 3.2657
User	13.24 (0.07 – 0.23)	0.24 (± 0.69; ± 0.08)	0	0 – 3	0.0028 (± 0.02; ± 0.002)	0	0 – 0.1563
Sheetlike	4.41 (0.01 – 0.11)	0.06 (± 0.29; ± 0.04)	0	0 – 2	0.0001 (± 0.0004; ± 0.0001)	0	0 – 0.0031
Threadlike	2.94 (0.01 – 0.09)	0.06 (± 0.38; ± 0.05)	0	0 – 3	0.00004 (± 0.0003; ± 0.00004)	0	0 – 0.0028
Foam	1.47 (0.002 – 0.07)	0.01 (± 0.12; ± 0.01)	0	0 – 1	0.00002 (± 0.002; ± 0.00002)	0	0 – 0.0015
Fragments	2.94 (0.01 – 0.09)	0.03 (± 0.17; ± 0.02)	0	0 – 1	0.0002 (± 0.001; ± 0.0001)	0	0 – 0.0084
Other	5.88 (0.02 – 0.13)	0.07 (± 0.31; ± 0.04)	0	0 – 2	0.0024 (± 0.02; ± 0.002)	0	0 – 0.1563

Table 3.10: Data on the plastics ingested by *Larus michahellis* (n = 96) based on plastics categories. Frequency of occurrence of plastics (with Jeffery's nominal 95% confidence intervals – CI) and plastic litter abundance. Abundance was calculated including all individuals sampled (affected and non-affected).

	Frequency of occurrence (%; 95% CI)	Number of plastic items			Mass of plastic items		
		Mean (n; ± sd; ± se)	Median	Range	Mean (g; ± sd; ± se)	Median	Range
Global	8.33 (0.04 – 0.15)	0.17 (± 0.74; ± 0.08)	0	0 – 6	0.0019 (± 0.01; ± 0.001)	0	0 – 0.0766
Industrial	0 (0 – 0.03)	0	0	0	0	0	0
User	8.33 (0.04 – 0.15)	0.17 (± 0.74; ± 0.08)	0	0 – 6	0.0019 (± 0.01; ± 0.001)	0	0 – 0.0766
Sheetlike	3.12 (0.01 – 0.08)	0.03 (± 0.17; ± 0.02)	0	0 – 1	0.0003 (± 0.003; ± 0.0003)	0	0 – 0.0317
Threadlike	2.08 (0.004 – 0.07)	0.04 (± 0.32; ± 0.03)	0	0 – 3	0.00002 (± 0.0002; ± 0.00002)	0	0 – 0.0016
Foam	0 (0 – 0.03)	0	0	0	0	0	0
Fragments	3.12 (0.01 – 0.08)	0.08 (± 0.63; ± 0.06)	0	0 – 6	0.0007 (± 0.005; ± 0.0005)	0	0 – 0.0379
Other	1.04 (0.001 – 0.05)	0.01 (± 0.10; ± 0.01)	0	0 – 1	0.008 (± 0.01; ± 0.001)	0	0 – 0.0766

Table 3.11: Data on the plastics ingested by *Morus bassanus* (n = 18) based on plastics categories. Frequency of occurrence of plastics (with Jeffery's nominal 95% confidence intervals – CI) and plastic litter abundance. Abundance was calculated including all individuals sampled (affected and non-affected).

	Frequency of occurrence (%; 95% CI)	Number of plastic items			Mass of plastic items		
		Mean (n; ± sd; ± se)	Median	Range	Mean (g; ± sd; ± se)	Median	Range
Global	5.56 (0.01 – 0.23)	6.78 (± 28.76; ± 6.78)	0	0 – 122	0.0038 (± 0.02; ± 0.004)	0	0 – 0.0676
Industrial	0 (0 – 0.13)	0	0	0	0	0	0
User	5.56 (0.01 – 0.23)	6.78 (± 28.76; ± 6.78)	0	0 – 122	0.0038 (± 0.02; ± 0.004)	0	0 – 0.0676
Sheetlike	0 (0 – 0.13)	0	0	0	0	0	0
Threadlike	0 (0 – 0.13)	0	0	0	0	0	0
Foam	5.56 (0.01 – 0.23)	6.78 (± 28.76; ± 6.78)	0	0 – 122	0.0038 (± 0.02; ± 0.004)	0	0 – 0.0676
Fragments	0 (0 – 0.13)	0	0	0	0	0	0
Other	0 (0 – 0.13)	0	0	0	0	0	0

Table 3.12: Characterization of the plastics (size and colour) found in the seven species in study.

Species	Plastic size category (%)				Plastic colour (%)						
	Microplastic (1–5 mm)	Mesoplastic (>5–20 mm)	Macroplastic (>20–100 mm)	Megaplastic (>100 mm)	White	Black	Yellow	Green	Red	Blue	Mixed
Ciconia ciconia	0	56.25	43.75	0	37.50	50.00	12.50	0	0	0	0
Larus fuscus	88.07	10.09	1.83	0	10.09	86.24	2.75	0.92	0	0	0
Larus michahellis	18.75	68.75	12.50	0	81.25	6.25	0	6.25	0	0	6.25
Morus bassanus	100	0	0	0	100	0	0	0	0	0	0

Threadlike contributed the most in distinguishing juveniles' and adults (SIMPER, 27.09%), followed by fragments, sheetlike, other and industrial plastic categories (20.46%, 19.80%, 16.34% and 13.00%, respectively). When comparing juveniles' and sub-adults, foamed plastics (30.98%) was found to be the most relevant contributor in the dissimilarity, followed by industrial plastics with a similar proportion (30.29%), fragments, other and threadlike plastics (12.30%, 11.74% and 9.46%, respectively). When comparing adults and sub-adults, the plastic category that contributed the most in distinguishing the two age categories was foamed plastics (33.94%), followed by industrial, other, fragments and sheetlike plastics (31.41%, 12.91%, 9.81% and 7.96%). The plot generated from principal coordinate analyses (PCO) did not form strong groupings between age classes, however sub-adults seems to be relatively clustered (Figure 3.2). The first two axes explained 27.6% and 23.5% of the variation between ages. Pearson correlation vectors show that all age classes tend to ingest more sheetlike and threadlike plastics.

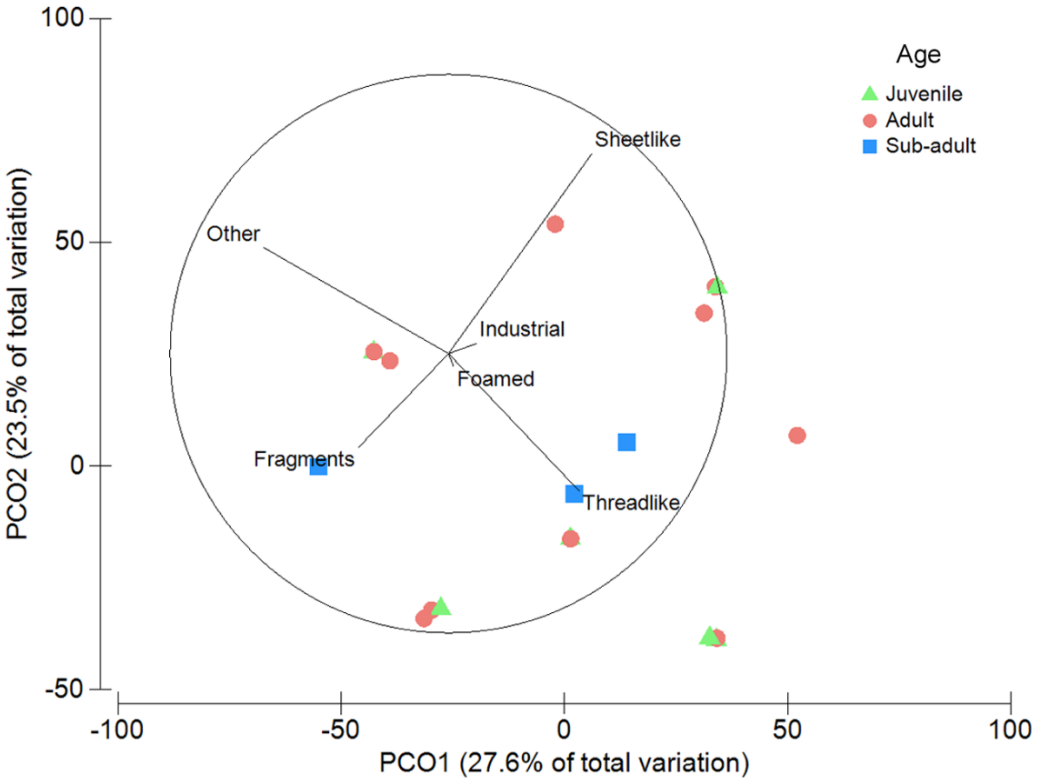

Figure 3.2: Principal coordinate analyses (PCO) based on plastic composition among age groups (dataset B). Black vector overlays represent Pearson's correlation coefficients of the dependent variables against the PCO axes. Vector length indicates strength of correlation. The size and position of origin (centre) of the circle is arbitrarily assigned with respect to the underlying plot.

Age had no effect on the plastic colour of the debris ingested (PERMANOVA, *P* (MC) = 0.586; Table 3.19) and there was no difference in dispersion among the three age groups (PERMDISP, *P* = 0.972).

When comparing juveniles' and adults, white coloured plastics contributed the most to distinguish these two groups (SIMPER, 48.20%), followed by black and yellow coloured plastics (24.52% and 19.37%, respectively). When comparing juveniles' and sub-adults, white coloured plastics (48.35%) was the most important contributor in the differentiation, followed by black coloured plastics with a similar proportion (47.20%). When comparing adults and sub-adults, the plastic colour that contributed the most to distinguish these age categories was black (49.51%), followed by white coloured plastics (43.09%). The PCO did not form strong groupings between age classes, however sub-adults seem to be relatively clustered (Figure 3.3). Samples appeared to form a gradient along PCO1, which described 47% of the variation, while the two first axes combined explained 78% of the variation. Pearson correlation vectors showed that all age classes tend to ingest more white and black coloured pieces of plastics.

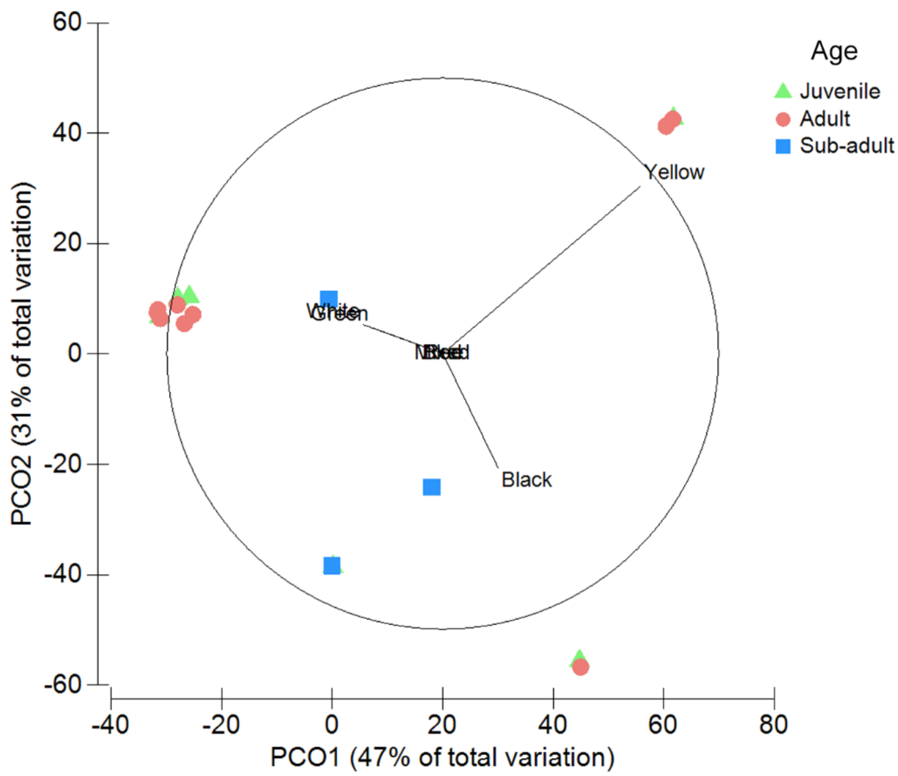

Figure 3.3: Principal coordinate analyses (PCO) based on plastic composition among age classes (dataset B). Superimposed black vectors represent Pearson's correlation coefficient of the dependent variables against the PCO axes. Vector length indicates strength of correlation. The circle size and position of origin (centre) is arbitrarily assigned with respect to the underlying plot.

Males and females did not ingest significant different types of plastics (PERMANOVA, *P* (MC) = 0.443; Table 3.20) and variability between genders was not significantly different (PERMDISP, *P* = 0.729).

Fragments (SIMPER, 22.72%) contributed the most to the differences between genders, followed by industrial, sheetlike, other and threadlike plastics (19.58%, 18.80%, 17.23% and 15.73%, respectively). The PCO generated did not form strong groupings between genders (Figure 3.4). In the PCO, the first two axes explained 34.1% and 23.6% of the variation between males and females.

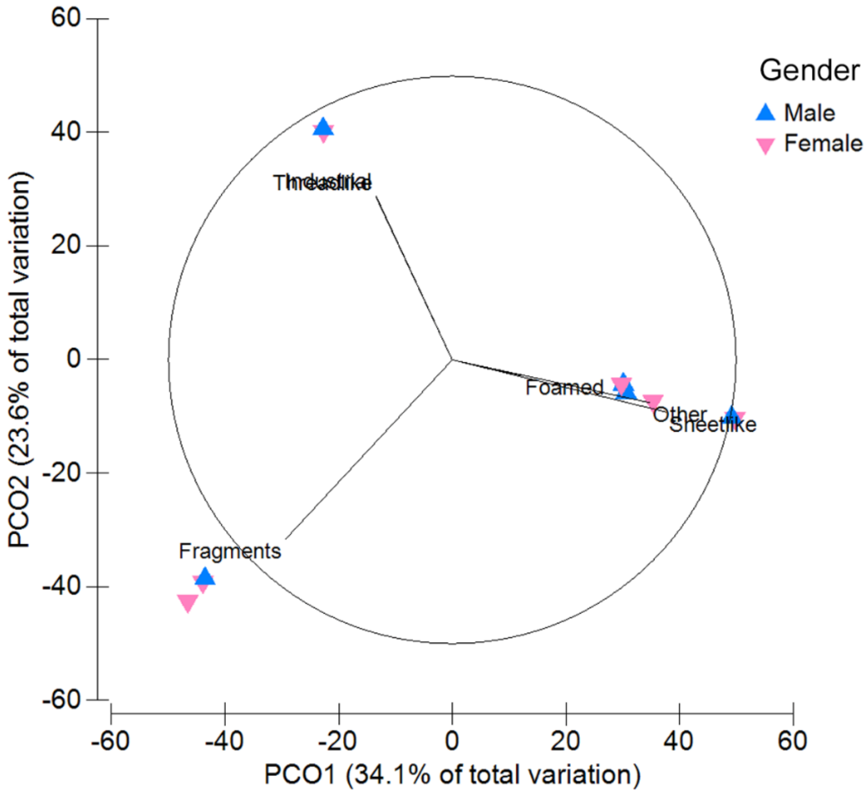

Figure 3.4: Principal coordinate analyses (PCO) based on plastic composition between genders (dataset B). Overlaid black vectors represent Pearson's correlation coefficients of the dependent variables against the PCO axes. Correlation strength is indicated by the vector length. The size and position of origin (centre) of the circle is arbitrarily assigned with respect to the underlying plot.

Males and females did not significantly ingest plastic debris of different colours (PERMANOVA, *P* (MC) = 0.519; Table 3.21). The variability of plastic colour did not significantly differ between genders (PERMDISP, *P* = 0.289).

White coloured plastics (SIMPER, 42.89%) contributed the most to the differences between genders, followed by black and yellow (25.46% and 25.36%, respectively). The plot generated from PCO did not form strong groupings, although males and females seem to be relatively clustered (Figure 3.5). The first two axes explained 56.1% and 38.6% of the variation between genders. Pearson correlation vectors show that males tend to ingest mostly white and yellow coloured plastics debris, while females tend to ingest mainly white and black coloured plastics.

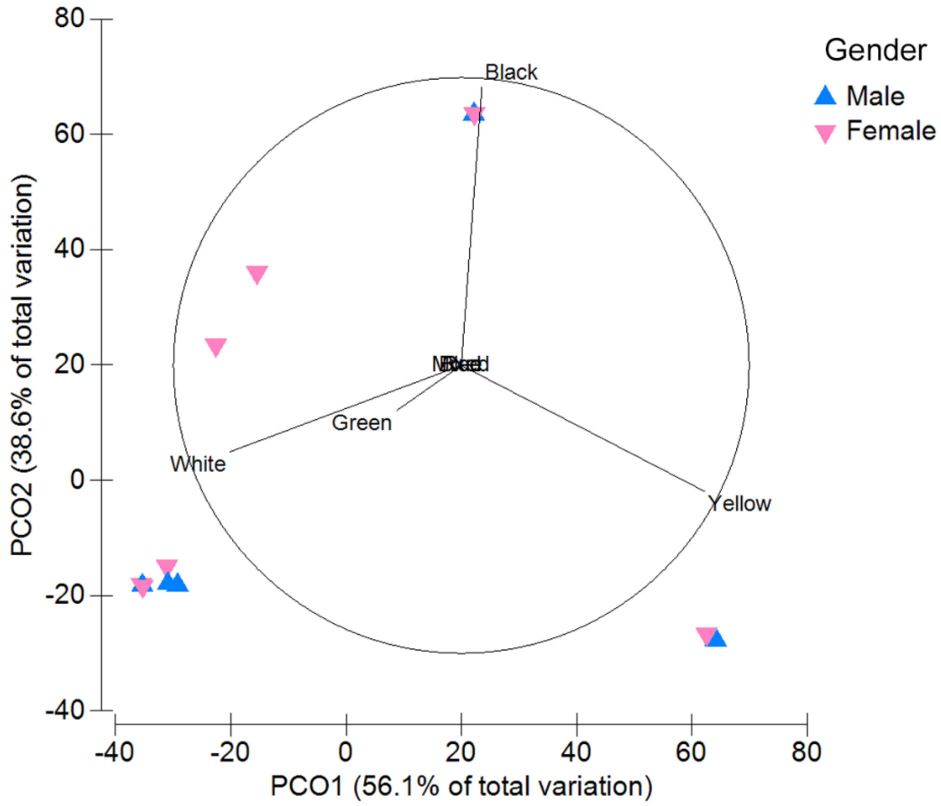

Figure 3.5: Principal coordinate analyses (PCO) based on plastic composition between genders (dataset B). Black vectors overlaid represent Pearson's correlation coefficients of the dependent variables against the PCO axes. Vector length indicates strength of correlation. The size and position of origin (centre) of the circle is arbitrarily assigned with respect to the underlying plot.

Moreover, there was no significant correlation between body condition of birds and the number (Pearson's R = -0.2596, P = 0.2558, R^2 = 0.0674; Figure 3.6A) or mass of plastic items measured (Pearson's R = -0.1744, P = 0.4497, R^2 = 0.0304; Figure 3.6B).

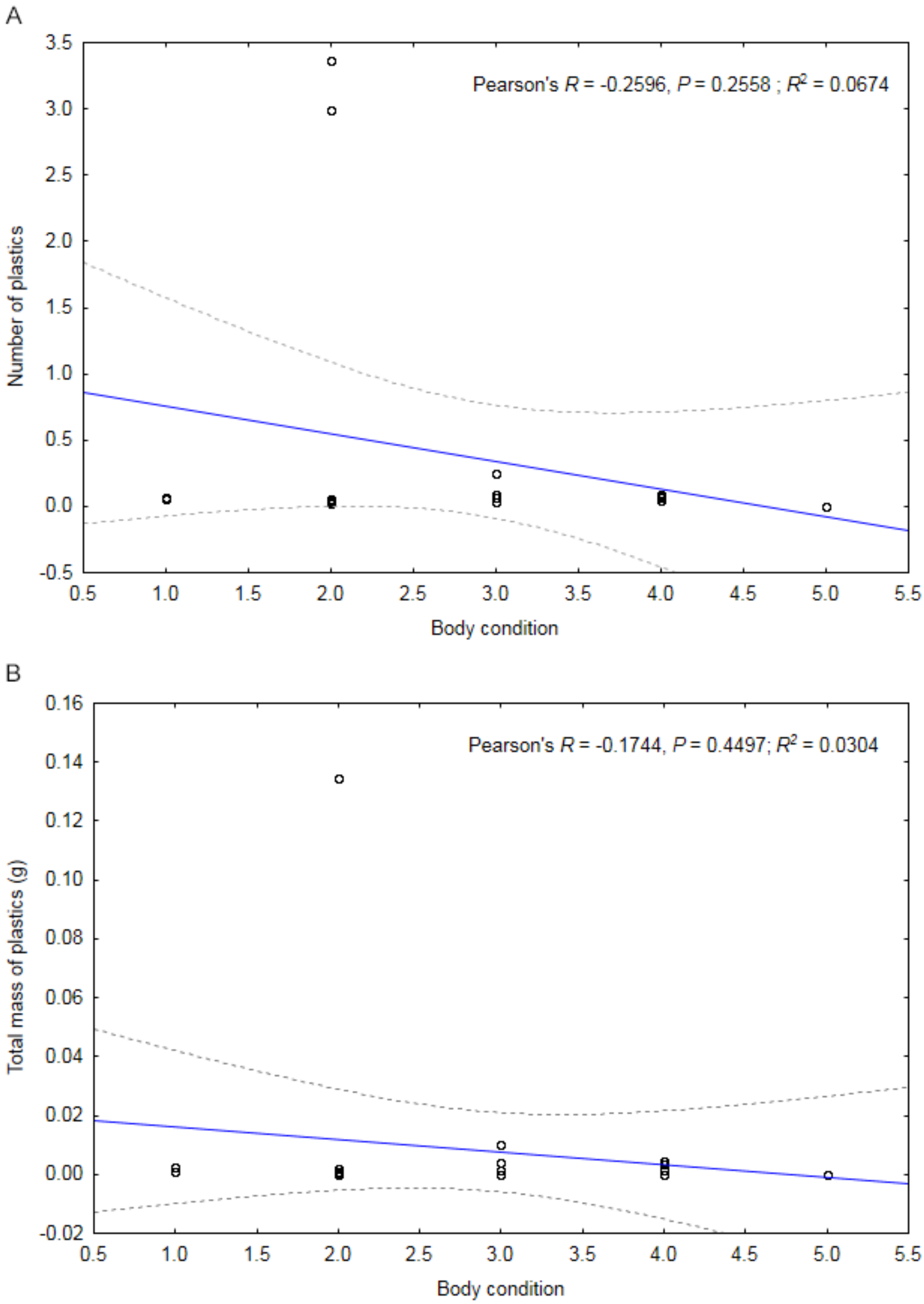

Figure 3.6: Relation between body condition of the bird and the amount (A) and total mass (B) of plastics ingested.

Table 3.13: PERMANOVA results of the model computed to test for differences on the number of plastic debris ingested among four different age classes (i.e., chick, juvenile, sub-adult and adult). Significance level was set as < 0.05.

Source	df	SS	MS	Pseudo - *F*	*P* (perm)	Unique perms	*P* (MC)
Age	3	401.5	133.83	3.4402	0.05	999	0.018
Residual	155	6029.9	38.903				
Total	158	6431.4					

Table 3.14: PERMANOVA pairwise test for the significant main effect age in Table 3.13.

Group	t	*P* (perm)	Unique perms	*P* (MC)
Juvenile, Adult	0.10932	0.936	992	0.913
Juvenile, Sub-adult	2.2693	0.03	205	0.032
Juvenile, Chick	0.53654	1	17	0.587
Adult, Sub-adult	2.6113	0.022	424	0.012
Adult, Chick	0.63864	0.937	38	0.518
Sub-adult, Chick	0.67261	0.879	7	0.532

Table 3.15: PERMANOVA results of the model computed to test for differences on the total mass of plastic debris ingested among four different age classes (i.e., chick, juvenile, sub-adult and adult). Significance level was set as < 0.05.

Source	df	SS	MS	Pseudo - *F*	*P* (perm)	Unique perms	*P* (MC)
Age	3	12.978	4.326	2.372	0.069	999	0.079
Residual	155	282.68	1.8238				
Total	158	295.66					

Table 3.16: PERMANOVA results of the model computed to test for differences on the number of plastic debris ingested between genders. Significance level was set as < 0.05.

Source	df	SS	MS	Pseudo - *F*	*P* (perm)	Unique perms	*P* (MC)
Gender	1	0.78462	0.78462	0.0481	0.841	921	0.851
Residual	95	1551	16.326				
Total	96	1551.1					

Table 3.17: PERMANOVA results of the model computed to test for differences on the total mass of plastic debris ingested between genders. Significance level was set as < 0.05.

Source	df	SS	MS	Pseudo - *F*	*P* (perm)	Unique perms	*P* (MC)
Gender	1	0.0699	0.0699	0.109	0.749	935	0.768
Residual	95	60.687	0.63881				
Total	96	60.757					

Table 3.18: PERMANOVA results of the model computed to test for differences in the type of plastic debris ingested among three different age classes (i.e., juvenile, sub-adult and adult). Significance level was set as < 0.05.

Source	df	SS	MS	Pseudo - *F*	*P* (perm)	Unique perms	*P* (MC)
Age	2	4704	2352	0.542	0.897	999	0.844
Residual	16	69384	4336.5				
Total	18	74088					

Table 3.19: PERMANOVA results of the model computed to test for differences in the colour of plastic debris ingested among three different age classes (i.e., juvenile, sub-adult and adult). Significance level was set as < 0.05.

Source	df	SS	MS	Pseudo - *F*	*P* (perm)	Unique perms	*P* (MC)
Age	2	4634.5	2317.3	0.794	0.566	998	0.586
Residual	16	46710	2919.4				
Total	18	51344					

Table 3.20: PERMANOVA results of the model computed to test for differences in the type of plastic debris ingested between genders. Significance level was set as < 0.05.

Source	df	SS	MS	Pseudo - *F*	*P* (perm)	Unique perms	*P* (MC)
Gender	1	3866.1	3866.1	0.9313	0.483	566	0.443
Residual	11	45664	4151.3				
Total	12	49530					

Table 3.21: PERMANOVA results of the model computed to test for differences in the colour of plastic debris ingested between genders. Significance level was set as < 0.05.

Source	df	SS	MS	Pseudo - *F*	*P* (perm)	Unique perms	*P* (MC)
Gender	1	2113.5	2113.5	0.679	0.491	765	0.519
Residual	11	34219	3110.8				
Total	12	36333					

3.3.Dataset C

For dataset C, a total of 58 individuals from the species *Ciconia ciconia* were included. Of these 58 individuals, 15 (frequency of occurrence of 25.86%) had ingested plastic debris, with an average number of 1.41 (± 4.97 SD) pieces of plastic litter per bird, an average mass of 0.2441 g (± 1.09 SD) and an average length of plastic particle of 1.98 mm (± 12. 57 SD).

Table 3.22: Data on the plastics ingested by *Ciconia ciconia* from northern regions (n = 47) based on plastics categories. Frequency of occurrence of plastics (with Jeffery's nominal 95% confidence intervals – CI) and plastic litter abundance. Abundance was calculated including all individuals sampled (affected and non-affected).

	Frequency of occurrence (%; 95% CI)	Number of plastic items			Mass of plastic items		
		Mean (n; ± sd; ± se)	Median	Range	Mean (g; ± sd; ± se)	Median	Range
Global	21.28 (0.12 – 0.34)	1.38 (± 5.42; ± 0.79)	0	0 – 35	0.0354 (± 0.16; ± 0.02)	0	0 – 1.0145
Industrial	0 (0 – 0.05)	0	0	0	0	0	0
User	21.28 (0.12 – 0.34)	1.38 (± 5.42; ± 0.79)	0	0 – 35	0.0354 (± 0.16; ± 0.02)	0	0 – 1.0145
Sheetlike	12.77 (0.06 – 0.24)	0.28 (± 0.95; ± 0.14)	0	0 – 5	0.0031 (± 0.01; ± 0.002)	0	0 – 0.0864
Threadlike	4.26 (0.01 – 0.13)	0.17 (± 1.03; ± 0.15)	0	0 – 7	0.0227 (± 0.15; ± 0.02)	0	0 – 1.0145
Foam	4.26 (0.01 – 0.13)	0.04 (± 0.20; ± 0.03)	0	0 – 1	0.0011 (± 0.01; ± 0.001)	0	0 – 0.0483
Fragments	12.77 (0.06 – 0.24)	0.26 (± 0.94; ± 0.14)	0	0 – 6	0.0038 (± 0.01; ± 0.002)	0	0 – 0.0552
Other	4.26 (0.01 – 0.13)	0.64 (± 3.59; ± 0.52)	0	0 – 24	0.0024 (± 0.02; ± 0.002)	0	0 – 0.1115

Table 3.23: Data on the plastics ingested by *Ciconia ciconia* from southern regions (n = 11) based on plastics categories. Frequency of occurrence of plastics (with Jeffery's nominal 95% confidence intervals – CI) and plastic litter abundance. Abundance was calculated including all individuals sampled (affected and non-affected).

	Frequency of occurrence (%; 95% CI)	Number of plastic items			Mass of plastic items		
		Mean (n; ± sd; ± se)	Median	Range	Mean (g; ± sd; ± se)	Median	Range
Global	45.45 (0.20 – 0.73)	1.55 (± 2.34; ± 0.71)	0	0 – 6	0.8373 (± 2.27; ± 0.68)	0	0 – 7.6339
Industrial	0 (0 – 0.20)	0	0	0	0	0	0
User	45.45 (0.20 – 0.73)	1.55 (±2.34; ± 0.71)	0	0 – 6	0.8373 (± 2.27; ± 0.68)	0	0 – 7.6339
Sheetlike	9.09 (0.01 – 0.35)	0.36 (± 1.21; ± 0.36)	0	0 – 4	0.0323 (± 0.11; ± 0.03)	0	0 – 0.3551
Threadlike	0 (0 – 0.20)	0	0	0	0	0	0
Foam	0 (0 – 0.20)	0	0	0	0	0	0
Fragments	9.09 (0.01 – 0.35)	0.18 (± 0.60; ± 0.18)	0	0 – 2	0.0025 (± 0.01; ± 0.002)	0	0 – 0.0271
Other	36.36 (0.14 – 0.65)	1.00 (± 1.84; ± 0.56)	0	0 – 6	0.8017 (± 2.28; ± 0.7)	0	0 – 7.6339

Table 3.24: Characterization of the plastics (size and colour) found in the *Ciconia ciconia* from northern and southern regions.

Region	Plastic size category (%)				Plastic colour (%)						
	Microplastic (1–5 mm)	Mesoplastic (>5–20 mm)	Macroplastic (>20–100 mm)	Megaplastic (>100 mm)	White	Black	Yellow	Green	Red	Blue	Mixed
North	46.15	35.38	10.77	7.69	33.85	44.62	1.54	1.54	3.08	15.38	0
South	0	52.94	41.18	5.88	35.29	52.94	11.76	0	0	0	0

Ciconia ciconia from the South presented a higher frequency of occurrence in comparison to the North region (Table 3.22 and 3.23). When comparing the two regions, both recorded on average a similar number of plastic debris, but specimens from the southern region ingest on average heavier pieces of plastic.

Most of items found were categorized as user plastics. No industrial plastics were found to be ingest in either of the two regions. Within user plastics, other seem to be the sub-category most reported on both regions, followed by sheetlike, fragments, treadlike and foam. No threadlike and foam plastics were recorded on the South.

Microplastic was the most common size category ingested by *C. ciconia* from the northern region, while mesoplastics was the most common size category ingested by the specimens from the southern region (Table 3.24). *Ciconia ciconia* from both North and South regions mainly ingested black coloured plastics.

The univariate analyses performed on dataset C showed that there was no significant difference in the incidence of plastics ingested by *C. ciconia* from northern and southern regions (PERMANOVA, P (MC) < 0.05; Table 3.25) and the dispersion did not significantly differ between *C. ciconia* from North and South (PERMDISP, P = 0.318).

There was significant difference in the total mass of plastic litter by *C. ciconia* from northern and southern regions (PERMANOVA, P (MC) < 0.05; Table 3.26). There was significant difference in dispersion between *C. ciconia* that inhabit the two regions (PERMDISP, P = 0.004).

The multivariate analyses performed only on affected specimens from dataset C showed that there was significant difference in the type of plastic litter ingested by *C. ciconia* from northern and southern regions (PERMANOVA, P (MC) < 0.05; Table 3.27). The variability between the species that inhabit these regions was not significantly different (PERMDISP, P = 0.254).

Plastic category other (SIMPER, 44.97%) contributed the most to the differences between regions, followed by sheetlike and fragments (24.29% and 22.77%, respectively). The plot generated from PCO formed relatively strong groupings between regions (Figure 3.7). The first two axes explained 43.1% and 30.7% of the variation between regions. Pearson correlation vectors showed that southern samples tend to ingest more the plastic sub-category other, while northern samples tend to ingest more fragments and sheetlike plastics.

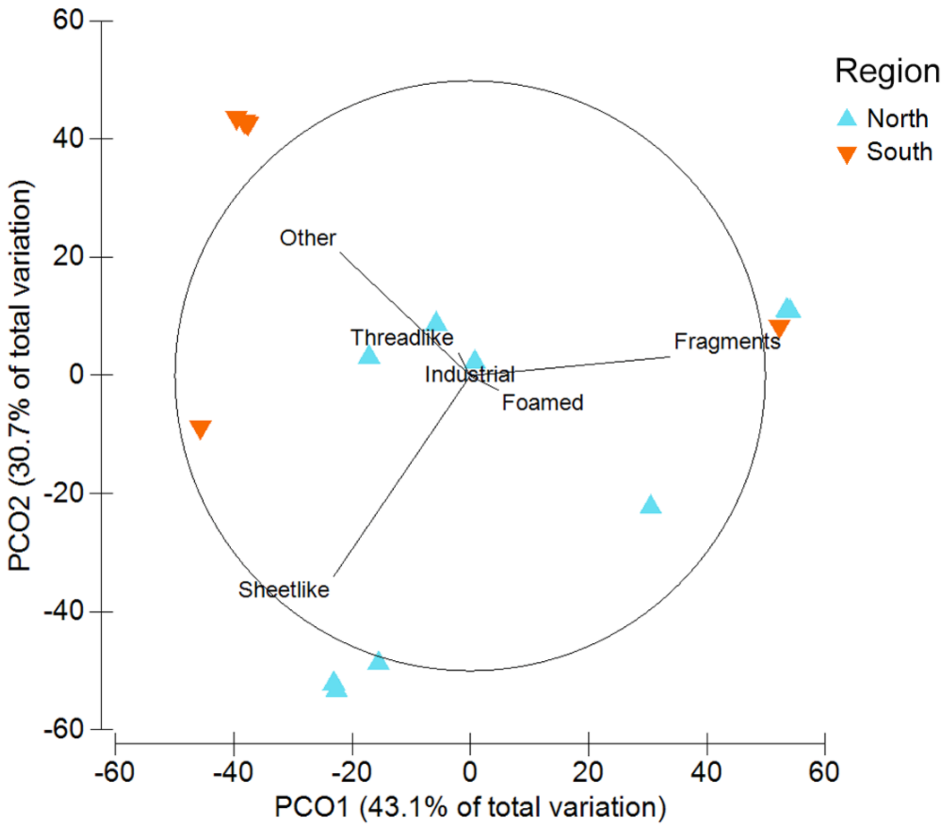

Figure 3.7: Principal coordinate analyses (PCO) based on plastic composition between regions (dataset C). Superimposed black vectors represent Pearson's correlation coefficient of the dependent variables against the PCO axes. Vector length indicates strength of correlation. The circle size and position of origin (centre) is arbitrarily assigned with respect to the underlying plot.

There was no significant difference in the colour of plastic debris ingested by *C. ciconia* from North and South regions (PERMANOVA, *P* (MC) = 0.093; Table 3.28). The variability of plastic colour did not differ between specimens from the different regions (PERMDISP, *P* = 0.868).

Black coloured plastics (SIMPER, 37.74%) contributed the most to the differences between *C. ciconia* from North and South regions followed by white, yellow and blue coloured plastics (33.71%, 13.02% and 10.18%, respectively). The plot generated from PCO formed relatively strong groupings between regions (Figure 3.8). The first two axes explained 37.6% and 23.4% of the variation between regions. Pearson correlation vectors showed that southern samples tend to ingest mostly black coloured plastics, while northern samples tend to ingest mainly white coloured plastic debris.

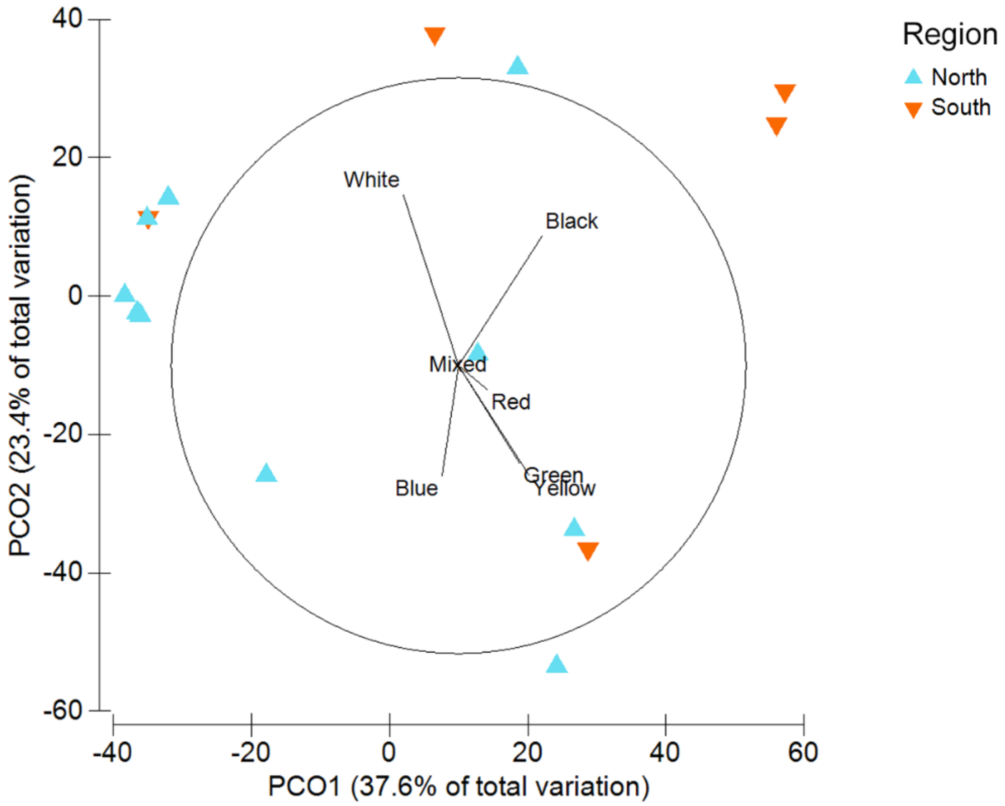

Figure 3.8: Principal coordinate analyses (PCO) based on plastic composition between regions (dataset C). Overlaid black vectors represent Pearson's correlation coefficients of the dependent variables against the PCO axes. Correlation strength is indicated by thee vector length. The size and position of origin (centre) of the circle is arbitrarily assigned with respect to the underlying plot.

Overall, polydimethylsiloxane (PDMS) was the polymer that C. ciconia ingested the most, followed by polystyrene (PS), polyethylene (PE), polyamide (PA) and polypropylene (PP; Table 3.29). Between regions, PDMS was the polymer type most ingested. There was no significant difference in the plastic polymers ingested by C. ciconia from the two regions (PERMANOVA, P (MC) = 0.582; Table 3.30) and the variability between the two groups was not significantly different (PERMDISP, P = 0.248).

PDMS (SIMPER, 37.24%) was the polymer that contributed the most to the differences between C. ciconia from northern and southern regions, followed by PE, PS and PA (25.99%, 19.94% and 8.66%, respectively). The plot generated from PCO did not form strong groupings (Figure 3.9). The first two axes explained 42.4% and 34.3% of the variation between regions. Pearson correlation vectors showed that southern samples tend to ingest more PDMS, while northern samples tend to ingest more PS plastic.

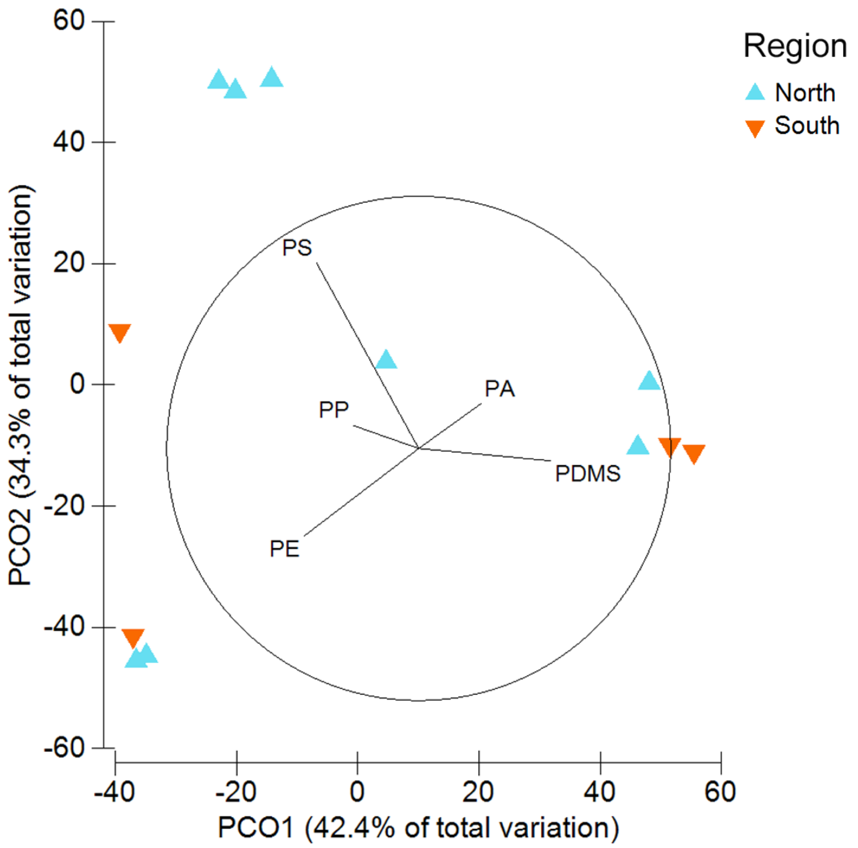

Figure 3.9: Principal coordinate analyses (PCO) based on plastic composition between regions (dataset C). Overlaid black vectors represent Pearson's correlation coefficients of the dependent variables against the PCO axes. Correlation strength is indicated by the vector length. The size and position of origin (centre) of the circle is arbitrarily assigned with respect to the underlying plot.

The overall number as well as the total mass of plastic items per affected bird stomach has increased over the study period at a non-significant level (Figure 3.10). This was particularly evident for total mass of plastics ingested.

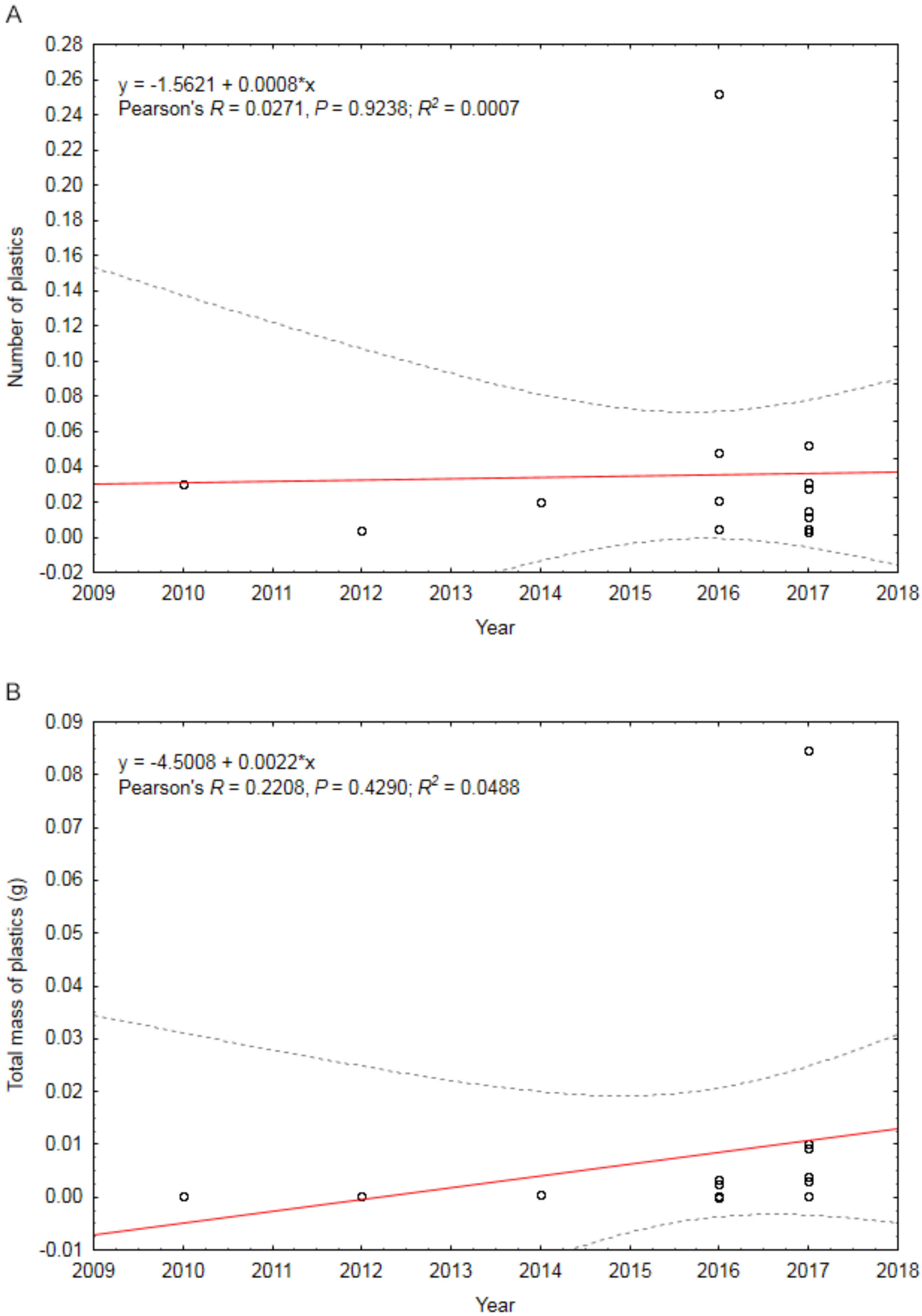

Figure 3.10: Trends over time in (A) number and (B) total mass of plastic items for *Ciconia ciconia* over the period 2010 – 2017.

Table 3.25: PERMANOVA results of the model computed to test for differences in the incidence of plastic debris ingested between regions. Significance level was set as < 0.05.

Source	df	SS	MS	Pseudo - *F*	*P* (perm)	Unique perms	*P* (MC)
Region	1	36.838	36.838	2.6858	0.093	599	0.103
Residual	56	768.08	13.716				
Total	57	804.92					

Table 3.26: PERMANOVA results of the model computed to test for differences in the total mass of plastic debris ingested between regions. Significance level was set as < 0.05

Source	df	SS	MS	Pseudo - *F*	*P* (perm)	Unique perms	*P* (MC)
Region	1	31.893	31.893	9.7891	0.007	627	0.002
Residual	56	182.45	3.258				
Total	57	214.34					

Table 3.27: PERMANOVA results of the model computed to test for differences in the type of plastic debris ingested between regions. Significance level was set as < 0.05.

Source	df	SS	MS	Pseudo - *F*	*P* (perm)	Unique perms	*P* (MC)
Region	1	8985.3	8985.3	3.0315	0.027	862	0.027
Residual	13	38532	2964				
Total	14	47517					

Table 3.28: PERMANOVA results of the model computed to test for differences in the colour of plastic debris ingested between regions. Significance level was set as < 0.05.

Source	df	SS	MS	Pseudo - *F*	*P* (perm)	Unique perms	*P* (MC)
Region	1	6275.8	6275.8	2.095	0.08	851	0.093
Residual	13	38942	2995.6				
Total	14	45218					

Table 3.29: Characterization of the plastic polymers found in *Ciconia ciconia* species from North and South regions. Abbreviations stand for the polymers found, namely polydimethylsiloxane (PDMS), polystyrene (PS), polyethylene (PE), polyamide (PA) and polypropylene (PP).

Region	Number of plastics found	Plastic polymer %				
		PDMS	PS	PE	PA	PP
Global	82	47.56	10.98	15.85	14.63	3.66
North	65	47.69	13.85	16.92	12.31	1.54
South	17	47.06	0	11.76	23.53	11.76

Table 3.30: PERMANOVA results of the model computed to test for differences in the polymer of plastic debris ingested between regions. Significance level was set as < 0.05.

Source	df	SS	MS	Pseudo - *F*	*P* (perm)	Unique perms	*P* (MC)
Region	1	2475.2	2475.2	0.666	0.616	421	0.582
Residual	10	37170	3717				
Total	11	39645					

4. Discussion

This study provides baseline data on plastic ingestion in a total of nineteen aquatic bird species in Portugal. In this study, I report evidence of plastic ingestion in four species. Results show that the frequency of plastic occurrence in Laridae are similar to the ones reported in northern and southern Europe (Acampora et al., 2016; Codina-García et al., 2013). In southern Portugal, particularly in the region of Algarve (Nicastro et al., 2018), higher frequencies of plastic occurrence were found in both species L. fuscus and L. michahellis when results are compared to the ones obtained in this study and in the same region (dataset B). Conversely to other works that reported comparatively high frequencies of plastic occurrence in M. bassanus (i.e., Acampora et al., 2016; Codina-García et al., 2013; Kühn et al., 2015), of the 21 individuals processed in this study only one had ingested plastic debris. This species also has a characteristic plunge diving fishing method that leads to higher rates of entanglement, mainly because individuals mistake floating plastic debris for fish or other food (Rountree, 1989). Ciconia ciconia are the species with higher frequency of plastics ingested, yet the levels reported in this study are lower when compared to previous studies in the Iberian Peninsula (i.e., Peris, 2003).

Several studies have shown that propensity of a species to ingest plastic is expected to vary according to foraging strategies (i.e., Azzarello and Vleet, 1987; Ryan, 1988a; Ryan, 1988b; Shephard et al., 2015). For example, several gull species are particularly exposed to the risk of ingesting plastic waste because, in addition to foraging in marine habitats, they feed from land-based sources including general public litter, industry, harbours and unprotected landfills and dumps located near the coast (Belant et al., 1998; Duhem et al., 2003; Lindborg et al., 2012; Seif et al., 2017). In fact, it has been shown that some gulls may specialise on landfills (Bond, 2016; Weiser and Powell, 2011). Ciconia ciconia is a species with an opportunist diet, feeding on whatever is available; howbeit, it's natural diet is entirely animal (del Hoyo et al., 1992). Earthworms (Lumbricidae) compose a large part of this species diet (Antczak et al., 2002), and mostly because of the similar shape and colour that mimic this prey, rubber bands are reported as one of the most common anthropogenic debris ingested by this species and other birds foraging on worms (Figure 4.1; Henry et al., 2011). Albeit, since it is not possible to determine whether the plastic particles present in the stomach contents of C. ciconia originates from anthropogenic habitats, there is ample evidence that landfills have also become an important food source for the European *C. ciconia* (Antczak *et al.*, 2002; Gilbert *et al.*, 2016; Peris, 2003; Rosa *et al.*, 2009; Tortosa *et al.*, 2002). This type of plastic debris can not only be detrimental if ingested, but also when incorporated into the nest structure (Kwieciński *et al.*, 2006). Rubber bands can be dangerous to chicks as they can become entangled and damage their legs (Kwieciński *et al.*, 2006). Since terrestrial locomotion is of great importance for storks when foraging (van Coppenolle and Aerts, 2004), even a small leg injury can put them at a disadvantage and negatively affect their chances of survival (Kwieciński *et al.*, 2006).

The European Union Landfill Directive (1993/31/EC) set a target to gradually reduce the volume of biodegradable municipal waste entering landfills until 2016, replacing open-air landfills by covered waist facilities of difficult access to birds (Gilbert *et al.*, 2016). Presently, in Portugal more than one third of plastic waste ends up in landfills (PlasticsEurope, 2016); thus, it is likely that, in a close future, the European Union Landfill Directive will lead to important consequences for aquatic birds in Portugal. For the Iberian *C. ciconia*, it is likely that this type of facilities eased the establishment of resident individuals in a previously solely migratory species, meaning that in a close future there will be a harsh reduction in food waste availability which will have important consequences for this species (Rosa *et al.*, 2009).

It is important to note that gulls regurgitate large quantities of debris ingested, thus the assessment of stomach contents only represent a snapshot of ingestion. However, even if gulls are able to regurgitate indigestible items, the release of chemical contaminants from ingested plastic may have sub-lethal effects on physiology and behaviour (i.e., Henriksen *et al.*, 2000; Sagerup *et al.*, 2009).

Figure 4.1: Example of a stomach content of an adult specimen of *Ciconia ciconia*. Three of the five subcategories of user plastics (sheetlike, fragment and others) were retrieved. The more elongated plastics may resemble a living prey, such as earthworms.

Results have shown no significant differences in the amount and total mass of plastic debris ingested among the different species. Yet, significant differences were found in the amount of plastic litter ingested among the different age classes (i.e., chicks, juveniles, sub-adults and adults). Adults ingested more plastic litter by count than juveniles and sub-adults, which was not expected since young birds may be more prone to ingest plastic debris once they are naïve consumers and might still be carrying debris fed to them by their parents before fledging as previously reported by other authors (Acampora *et al.*, 2014; Carey, 2011; Rodríguez *et al.*, 2012). No significant differences were found in the total mass of plastics among ages. Also, no significant differences were found in the amount and total mass of plastic debris ingested between genders, meaning that differences between parental duties did not influence plastic ingestion. Between regions no differences were found in the incidence of plastic litter, although, significant differences were found in the total mass of plastics ingested. Differences in the types of plastic ingested by *C. ciconia* from northern and southern regions were observed, which can be due to differences in the types of plastic debris available in the environment. There are several types of plastic debris in Portuguese offshore waters, such as styrofoam, derelict or lost materials from fisheries and unidentified plastics (Sá *et al.*, 2016).

However, more studies on this subject using standardized methodologies must be developed to allow comparisons between the plastics present in the environment and the plastics ingested by aquatic birds. Caution should be taken as most of the northern samples in this study were collected inland, so it is possible that the different accumulation of types of plastic debris in the stomachs of *C. ciconia* from the distinct regions was due to the higher terrestrial and landfill foraging in individuals from northern areas.

Interestingly, no significant relationship was found between the number and total mass of plastic debris ingested and the body condition of the birds. This result is consistent with those of other authors who also did not detect a clear evidence of an effect on body condition of aquatic birds that had ingested plastic debris (Carey, 2011; Rodríguez *et al.*, 2012). In contrast, another study found a negative correlation between the number of particles and body condition indicators among the birds that had consumed plastics (Spear *et al.*, 1995). The same study reported higher plastic loads in heavier seabirds, further hypothesizing that birds with better body conditions are more prone to ingest plastic debris as they are more fit and can feed in different areas (Spear *et al.*, 1995).

Although not significant, time trends in the total mass of ingested plastic were increasing, emphasizing the importance of the continued monitoring of plastics in aquatic environments. This is in contrast with the trends for the total mass of plastic debris in Nederland since 1980s where a decrease in the mass of plastics ingested has been reported (van Franeker and Law, 2015; Van Franeker *et al.*, 2011).

Similar to other studies, the most common plastic type encountered was user plastics, with foam as the most common subtype (Acampora *et al.*, 2016), while some studies found fragments as the most common subtype of plastic debris (Codina-García *et al.*, 2013; Ryan, 2008; Van Franeker *et al.*, 2011). The low presence of industrial plastics in stomach contents is consistent with the findings in long term studies, where a decrease in this type of plastic in beaches and stomachs was detected since the 1980s (Ryan *et al.*, 2009; Van Franeker *et al.*, 2011). Although previous data on long-time monitoring studies in the Portuguese coast is not available, these findings might suggest that policy measures to reduce the input of plastic litter into the environment have been somewhat effective. There are several international and regional agreements that aimed to reduce the impacts of plastic litter, including the International Convention for the Prevention of Pollution

From Ships (MARPOL) Annex V 1978 with the latest amendment in 2012, the Convention on Biological Diversity (CDB, COP 11 Decision XI/18), the EU Marine Strategy Framework Directive (MSFD; 2008/56/EC) and the United Nations (UN) Sustainable Development Goals (SDG; UNDP, 2015). Microplastics were the most common size category reported in this study, indicating that smaller plastic particles do become more bioavailable and have a higher chance of being accidentally or selectively ingested than larger items (Lusher *et al.*, 2015). Silicones (PDMS) was the most common polymer type ingested by *C. ciconia* specimens from northern and southern regions as recently reported in the Algarve region (Nicastro *et al.*, 2018).

When looking at the biology of *M. bassanus*, this species can be selected as a good candidate for monitoring marine plastic litter in Portugal, since it is a strictly marine species that forages mainly over continental shelves. However, in order for a species to be considered a good bioindicator, some aspects have to be taken into account, including: (1) monitoring location, offshore or coastal as it will define what species can be considered, (2) local species abundance, through either breeding pairs or migration routes, (3) stranding occurrence and (4) probable accumulation of ingested aquatic litter (Acampora *et al.*, 2016). Additionally, some areas can be of difficult access, hence restricting sampling efforts or the presence of scavengers can decrease carcass availability (Acampora *et al.*, 2016). From my study, it is clear that *M. bassanus* does not fulfil the requisites needed to be considered as a candidate; of the 21 birds collected only one had ingested plastic debris, which lead to a low percentage frequency of occurrence and, consequently, a low probability of accumulation of ingested debris.

During this study, it became increasingly clear the need to stablish standardize dissection protocols and metrics when reporting ingested plastics in Portugal. While working with the several WRC around the country, it was possible to observe that some of them had their own protocol to proceed with the dissections or that dissections were not a priority. As described in the Materials and Methods chapter, body condition was assessed by palpation of the pectoral muscles (Carrega, 2016). This method was used since it was common between the wildlife rescue centres that performed dissections regularly. However, this methodology does not consider two significant characteristics of birds; (1) between species exists differences in the amount of the pectoral musculature, particularly between flighted and nonflighted birds and (2) fat does not only accumulates in this region, it also deposits in the coelom, over the flanks, around the thoracic inlet, on the back of the neck and on the back near the tale (Samour, 2000).

Van Franeker and Meijboom (2002) and Van Franeker (2004) developed a standard dissection protocol for the Northern fulmar (*Fulmarus glacialis*), where body condition is recorded considering the amount of subcutaneous fat, intestinal fat and the condition of the pectoral muscle by scoring them from 0 to 3, being 0 complete absence and 3 optimal condition. The sum of these three scores will then provide the overall condition index that can be divided in mortally emaciated (0 – 1), critically emaciated (2 – 3), moderate body condition (4 – 6) and good body condition (7 - 9; Van Franeker, 2004; Van Franeker and Meijboom, 2002). Several studies have applied this methodology successfully in their studies (Acampora *et al.*, 2014; Acampora *et al.*, 2016; Acampora *et al.*, 2017; Codina-García *et al.*, 2013; Kain *et al.*, 2016; Law *et al.*, 2010; van Franeker and Law, 2015; Van Franeker *et al.*, 2011). The initial objective of this study was to apply this methodology, however samples of aquatic birds that had already been dissected and consequently assess their body condition, did not allow the application of the desired methodology. In an attempt to be able to use the body condition index described by Van Franeker and Meijboom (2002), a proxy of the methodologies was made, where the values of the body condition used by most of the WRC were duly transformed for Van Franeker and Meijboom (2002) values of body condition index. This proxy has shown that such transformation was not possible, although the correlation was positive and significant, it was poorly supported.

The collection of data on plastic ingestion while performing the necropsy of whole specimens was a major advantage since it allowed the determination of age, gender, probable cause of death and body condition of the birds. This approach also allows the examination of the entire gastrointestinal tract for plastics, providing a certain level of certainty in the findings (Provencher *et al.*, 2017). However, the examination of the entire gastrointestinal tract was not performed in this study, since some of the birds arrived to the centres in an advanced stage of degradation, not allowing the collection of all gastrointestinal tract (Provencher *et al.*, 2017). There are other methods of sampling that can be also advantageous as they do not rely on opportunistic sampling; sampling live birds can be done systematically, though it is unclear whether 100% of plastics ingested can be collected *via* natural or induced regurgitations (i.e., stomach flushing or chemical emetics; Provencher *et al.*, 2017).

5. Final remarks

As the presence of plastics continues to increase in aquatic environments, this data will provide a solid record of affected species and a basis for longer-term trends in plastic ingestion, particularly for Portuguese and southern Europe monitoring programs for which information is scares or non-existed. Furthermore, by adopting the newest recommendations for standardization of plastic quantification in macrofauna (i.e., Provencher *et al.*, 2017; Van Franeker *et al.*, 2011), I want to emphasise the importance of implementing these accepted protocols and standardized metrics when reporting plastic ingestion in affected organisms so to provide means of comparison among studies.

Governments should play an active role in addressing this problem by introducing legislation that will control sources of plastic debris (Li *et al.*, 2016). In Portugal, the EU Marine Strategy Framework Directive (MSFD; 2008/56/EC) was adopted, aiming to implement monitoring programs to regularly assess the state of the marine environment (Galgani *et al.*, 2013). Plastic industries could also play an important role in reducing plastic debris in the environment, since they could take responsibility for the end-of-life of their own plastic products by recycling them (Li *et al.*, 2016).

In terms of recommendations for future research, it is critical to continue this type of studies in Portugal to obtain results based on a higher number of samples from different species and to understand which species should be considered as good bioindicators to monitor aquatic plastic debris that has been ingested or present in the environment. Since the ingestion of plastic by birds and other aquatic animals has potential harmful effects, it is urgent to evaluate the effects on health, particularly in the case of endangered species.

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Supplemental material

Table S 1: Detailed sample description.

Wildlife Rescue Centre	Species	Entrance date	Local	Weight (g)	Probable cause of death	Body condition	Gender	Age
PBGaia	*Ardea cinerea*	Fev/06/2017	Paços de Ferreira	-	Cahexia	2	Male	-
	Larus michahellis	Fev/27/2017	Vila Nova de Gaia	754	Euthanasia	3	-	Adult
	Morus bassanus	Mar/05/2017	Miramar, Vila Nova de Gaia	1020	Unknown	3	Male	Adult
	Larus michahellis	Mar/07/2017	Vila Nova de Gaia	625	Euthanasia	1	Female	Juvenile
	Larus michahellis	Mar/11/2017	Espinho, Aveiro	-	Euthanasia	2	Female	Juvenile
	Larus michahellis	Mar/12/2017	Matosinhos, Porto	867	Enterotox	2	Male	Adult
	Larus michahellis	Mar/13/2017	Vila Nova de Gaia	680	Euthanasia	3	Female	Adult
	Larus michahellis	Mar/25/2017	Porto	850	Euthanasia	3	Female	Adult
	Larus fuscus	Mar/28/2017	Vila Nova de Gaia	660	Euthanasia	1	Female	Adult
	Larus michahellis	Mar/28/2017	Vila Nova de Gaia	555	Euthanasia	2	Female	Juvenile
	Larus michahellis	Mar/29/2017	Porto	-	Euthanasia	1	-	Juvenile

Table S1: (cont.)

Wildlife Rescue Centre	Species	Entrance date	Local	Weight (g)	Probable cause of death	Body condition	Gender	Age
PBGaia	*Larus michahellis*	Mar/29/2017	Porto	572	Euthanasia	2	Female	Juvenile
	Larus michahellis	Mar/30/2017	Leça da Palmeira, Porto	755	Euthanasia	4	Female	Adult
	Larus michahellis	Apr/05/2017	Pedroso, Vila Nova de Gaia	760	Euthanasia	5	Male	Adult
	Larus michahellis	Apr/06/2017	Vila Nova de Gaia	687	Unknown	4	Female	Adult
	Larus argentatus	Apr/17/2017	Massarelos, Porto	728	Euthanasia	1	Male	Sub-adult
	Larus michahellis	Apr/24/2017	Porto	800	Enterotox	2	Male	Adult
	Larus michahellis	May/01/2017	Porto	950	Euthanasia	3	Male	Sub-adult
	Larus michahellis	May/03/2017	Miramar, Vila Nova de Gaia	750	Euthanasia	2	Male	Adult
	Larus michahellis	May/05/2017	Porto	730	Euthanasia	4	Female	Juvenile
	Larus michahellis	May/06/2017	Pedroso, Vila Nova de Gaia	915	Enterotox	3	Male	Adult
	Morus bassanus	May/12/2017	Esmoriz, Ovar	1880	Virus	1	Male	Sub-Adult
	Larus michahellis	May/17/2017	Vila Nova de Gaia	915	Trauma	4	Male	Adult

Table S1: (cont.)

Wildlife Rescue Centre	Species	Entrance date	Local	Weight (g)	Probable cause of death	Body condition	Gender	Age
PBGaia	*Larus michahellis*	May/20/2017	Matosinhos, Porto	860	Internal haemorrhage	3	Female	Juvenile
	Larus michahellis	May/22/2017	Vila Nova de Gaia	710	Internal haemorrhage	2	Female	Juvenile
CERVAS	*Ardea cinerea*	Oct/26/2007	Vide, Seia	1200	Trauma	3	Male	-
	Ciconia ciconia	Jan/25/2010	Campo Maior, Portalegre	4000	Electrocution	4	Male	Adult
	Ciconia ciconia	Mar/24/2010	Montemor-o-Velho, Coimbra	2700	Collision with electric line	3	-	Adult
	Ciconia ciconia	Jul/29/2010	Almeida, Guarda	1838	Trauma	2	Female	Adult
	Ciconia ciconia	Aug/05/2010	Antanhol, Coimbra	2800	Collision with electric line	3	Female	Juvenile
	Ciconia ciconia	Aug/06/2010	Taveiro, Coimbra	2205	Collision with electric line	3	Female	Juvenile
	Ardea cinerea	Aug/27/2010	Ponte de Sor, Portalegre	1546	Trauma	4	Male	Juvenile
	Ardea cinerea	Nov/16/2010	Arganil, Coimbra	1003	Debility	2	Male	Juvenile
	Ciconia ciconia	Jun/02/2011	Sabugal, Guarda	2900	Trauma	3	-	Adult
	Ciconia ciconia	Jun/08/2011	Castelo de Vide, Portalegre	1807	Fell off the nest	3	-	Chick
	Ciconia ciconia	Oct/18/2011	Condeixa-a-Nova, Coimbra	2050	Electrocution	2	-	Juvenile

Table S1: (cont.)

Wildlife Rescue Centre	Species	Entrance date	Local	Weight (g)	Probable cause of death	Body condition	Gender	Age
CERVAS	*Ardea cinerea*	Oct/18/2011	Mortágua, Viseu	1269	Run over	3	-	Adult
	Ardea cinerea	Dec/05/2011	Sabugal, Guarda	1100	Gunshot	3	-	Juvenile
	Ciconia ciconia	Dec/08/2011	Figueira da Foz, Coimbra	4000	Collision with electric line	4	Male	Adult
	Larus fuscus	Dec/08/2011	Santa Clara, Coimbra	474	Debility	2	Female	Juvenile
	Ardea cinerea	Dec/17/2011	Nespreira, Gouveia	1054	Electrocution	3	Female	Adult
	Ardea cinerea	Mar/12/2012	Mogadouro, Bragança	1362	Trauma	4	Male	-
	Ciconia ciconia	Jul/13/2012	Figueira de Castelo Rodrigo, Guarda	3100	Trauma	3	Female	Juvenile
	Ardea cinerea	Sept/06/2012	Coimbra	1200	Unknown	3	Female	Adult
	Ardea cinerea	Jan/12/2013	Figueira de Castelo Rodrigo, Guarda	1063	Trauma	3	Male	Juvenile
	Ciconia ciconia	Jul/03/2013	Condeixa-a-Nova, Coimbra	3700	Fell off the nest	4	Male	Juvenile
	Larus fuscus	Sept/27/2013	Figueira da Foz, Coimbra	633	Debility	3	-	Juvenile
	Ardea cinerea	Dec/09/2013	Figueira de Castelo Rodrigo, Guarda	1074	Collision with a structure	3	-	Adult
	Rissa tridactyla	Mar/05/2014	Vila Nova de Poiares, Coimbra	218	Debility	3	Female	Adult

Table S1: (cont.)

Wildlife Rescue Centre	Species	Entrance date	Local	Weight (g)	Probable cause of death	Body condition	Gender	Age
CERVAS	*Ciconia ciconia*	Jun/03/2014	Guarda	1668	Poisoned	3	Female	Chick
	Ciconia ciconia	Jun/03/2014	Guarda	1240	Poisoned	3	-	Chick
	Ciconia ciconia	Jun/03/2014	Guarda	1457	Poisoned	3	-	Chick
	Ciconia ciconia	Jun/03/2014	Guarda	1626	Poisoned	3	-	Chick
	Ciconia ciconia	Jun/14/2014	Rochoso, Guarda	-	Poisoned	1	-	Chick
	Ciconia ciconia	Jun/14/2014	Rochoso, Guarda	-	Poisoned	1	-	Chick
	Ciconia ciconia	Jun/14/2014	Rochoso, Guarda	-	Poisoned	1	-	Chick
	Ciconia ciconia	Jul/08/2014	Cernache, Coimbra	2900	Collision with electric line	2	Male	Juvenile
	Ciconia ciconia	Jul/31/2014	Figueira da Foz, Coimbra	2400	Collision with electric line	2	Male	Juvenile
	Ardea cinerea	Sept/12/2014	Belmonte, Castelo Branco	1300	Gunshot	3	Male	Juvenile
	Platalea leucorodia	Oct/03/2014	Lousã, Coimbra	1296	Debility	2	Male	Juvenile
	Ardea cinerea	Oct/10/2014	Penela, Coimbra	1053	Electrocution	3	Female	Juvenile
	Ciconia ciconia	Feb/19/2015	Almeida, Guarda	2300	Poisoned	3	Female	Adult

Table S1: (cont.)

Wildlife Rescue Centre	Species	Entrance date	Local	Weight (g)	Probable cause of death	Body condition	Gender	Age
CERVAS	*Ciconia ciconia*	Feb/24/2015	Sabugal, Guarda	5000	Trauma	5	Female	Adult
	Ciconia ciconia	Mar/11/2015	Montemor-o-Velho, Coimbra	2700	Collision with electric line	3	Female	Adult
	Ciconia ciconia	May/17/2015	Figueira de Castelo Rodrigo, Guarda	2800	Trauma	3	Female	Adult
	Ciconia ciconia	Jul/25/2015	Pinhel, Guarda	1780	Fell off the nest	4	Male	Chick
	Ciconia ciconia	Aug/01/2015	Sabugal, Guarda	3200	Trauma	3	Male	Adult
	Ciconia ciconia	Mar/02/2016	Rochoso, Guarda	3900	Collision with electric line	4	Male	Adult
	Ciconia ciconia	Mar/03/2016	Coimbra	2887	Collision with structure	3	Male	Adult
	Ardea cinerea	Mar/15/2016	Vouzela, Viseu	1036	Gunshot	2	-	Juvenile
	Ciconia ciconia	Apr/18/2016	Celorico da Beira, Guarda	2364	Collision with electric line	3	Female	Adult
	Ciconia ciconia	May/02/2016	Rio Torto, Gouveia	2500	Trauma	2	Female	Juvenile
	Ciconia ciconia	Jun/29/2016	Condeixa-a-Nova, Coimbra	2883	Trauma	3	Female	Juvenile
	Ciconia nigra	Feb/17/2016	Trancoso, Guarda	1376	Debility	1	-	Juvenile
	Larus michahellis	Mar/22/2016	Figueira da Foz, Coimbra	518	Trauma	3	-	Juvenile

Table S1: (cont.)

Wildlife Rescue Centre	Species	Entrance date	Local	Weight (g)	Probable cause of death	Body condition	Gender	Age
CERVAS	*Ciconia ciconia*	Feb/20/2017	Boidobra, Covilhã	4300	Electrocution	4	Male	Adult
	Ciconia ciconia	Feb/20/2017	Boidobra, Covilhã	2625	Electrocution	3	Male	Adult
	Gavea stellata	Feb/24/2017	Praia da Aguda, Vila Nova de Gaia	1925	Unknown	3	-	Juvenile
	Alca torda	Feb/24/2017	Praia da Aguda, Vila Nova de Gaia	563	Unknown	3	-	-
	Melanitta nigra	Feb/24/2017	Praia da Aguda, Vila Nova de Gaia	1179	Unknown	3	-	Juvenile
	Melanitta nigra	Feb/24/2017	Praia da Aguda, Vila Nova de Gaia	979	Unknown	3	-	Juvenile
	Alca torda	Feb/24/2017	Praia da Aguda, Vila Nova de Gaia	563	Unknown	3	-	-
CERAS	*Ciconia ciconia*	Feb/02/2016	Cabeção, Évora	-	Electrocution	3	-	Adult
	Ciconia ciconia	Jan/04/2017	Fundão, Castelo Branco	4400	Trauma	4	Male	Adult
	Phalacrocorax carbo	Feb/08/2017	Montemor-o-Novo, Évora	1025	Trauma	1	-	Adult
	Ciconia ciconia	Fev/27/2017	Belmonte, Castelo Branco	3391	Intoxication	3	-	Adult
	Ciconia ciconia	Mar/01/2017	Malpica do Tejo, Castelo Branco	4000	Trauma	4	-	Adult
	Ciconia ciconia	Apr/26/2017	Marateca, Catelo Branco	4000	Intoxication	2	-	Adult

Table S1: (cont.)

Wildlife Rescue Centre	Species	Entrance date	Local	Weight (g)	Probable cause of death	Body condition	Gender	Age
CERAS	*Ciconia ciconia*	Apr/28/2017	Idanha-a-Nova, Castelo Branco	4000	Trauma	3	Male	Adult
	Ciconia ciconia	May/09/2017	Idanha-a-Nova, Castelo Branco	832	Trauma	1	Male	Chick
	Ciconia ciconia	May/16/2017	Castelo Branco	-	Euthanasia	1	-	Adult
	Ciconia ciconia	Jun/09/2017	-	2888	Internal injury	1	-	Chick
	Ciconia ciconia	Jun/15/2017	Idanha-a-Nova, Castelo Branco	-	Stuck on a nylon thread	1	-	Chick
	Ciconia ciconia	Jun/18/2017	Alcains, Castelo Branco	2907	Unknown	2	Male	Juvenile
	Ciconia ciconia	Jun/19/2017	Idanha-a-Nova, Castelo Branco	1648	Anorexia and infection	2	Male	Juvenile
	Ciconia ciconia	Jun/21/2017	Castelo Branco	-	Trauma	2	Female	Juvenile
	Ciconia ciconia	Jul/02/2017	Castelo Branco	3500	Trauma	3	Male	Juvenile
LxCRAS	*Larus fuscus*	Oct/07/2016	Almada, Setúbal	468	Pododermatitis V	2	Female	-
	Larus fuscus	Dec/29/2016	Lisboa	573	Trauma	2	Male	-
	Larus fuscus	Jan/11/2017	Almada, Setúbal	878	Biotoxins	3	Male	-
	Larus, fuscus	Jan/19/2017	Almada, Setúbal	718	Biotoxins	2	Male	-

Table S1: (cont.)

Wildlife Rescue Centre	Species	Entrance date	Local	Weight (g)	Probable cause of death	Body condition	Gender	Age
LxCRAS	*Larus fuscus*	Jan/21/2017	Lisboa	612	Trauma	3	-	-
	Larus fuscus	Jan/25/2017	Lisboa	-	Trauma	3	-	-
	Larus fuscus	Jan/27/2017	Lisboa	600	Trauma	1	-	-
	Larus fuscus	Jan/27/2017	Setúbal	-	Trauma	5	-	-
	Larus michahellis	Jan/28/2017	Lisboa	545	Trauma	2	Female	-
	Larus michahellis	Jan/29/2017	Lisboa	571	Trauma	2	Male	-
	Larus fuscus	Feb/01/2017	Lisboa	676	Trauma	2	Male	-
	Larus fuscus	Feb/01/2017	Lisboa	648	Trauma	2	Female	-
	Larus fuscus	Feb/04/2017	Lisboa	776	Trauma	3	-	-
	Larus fuscus	Feb/07/2017	Setúbal	748	Trauma	2	-	-
	Larus fuscus	Feb/09/2017	Lisboa	-	Trauma	3	-	-
	Larus michahellis	Feb/10/2017	Almada, Setúbal	-	Run over	3	Female	-
	Larus fuscus	Feb/11/2017	Lisboa	812	Trauma	3	-	-

Table S1: (cont.)

Wildlife Rescue Centre	Species	Entrance date	Local	Weight (g)	Probable cause of death	Body condition	Gender	Age
LxCRAS	*Larus fuscus*	Feb/13/2017	Amadora, Lisboa	813	Trauma	3	Male	-
	Larus ridibundus	Feb/15/2017	Vila Franca de Xira, Lisboa	141	Unknown	1	Female	-
	Larus michahellis	Feb/22/2017	Lisboa	802	Gunshot	2	-	-
	Larus fuscus	Feb/23/2017	Amadora, Lisboa	670	Trauma	3	Female	-
	Larus michahellis	Feb/25/2017	Almada, Setúbal	852	Biotoxins	2	Female	-
	Larus fuscus	Feb/27/2017	Almada, Setúbal	727	Biotoxins	3	-	-
	Larus fuscus	Mar/02/2017	Almada, Setúbal	594	Biotoxins	1	-	-
	Larus fuscus	Mar/04/2017	Almada, Setúbal	560	Trauma	1	-	-
	Larus fuscus	Mar/04/2017	Lisboa	779	Neurotoxic biotoxins	2	-	-
	Larus fuscus	Mar/05/2017	Lisboa	812	Trauma	2	-	-
	Larus fuscus	Mar/08/2017	Cascais, Lisboa	616	Trauma	3	-	-
	Larus fuscus	Mar/08/2017	Lisboa	-	Run over	3	-	-
	Larus fuscus	Mar/09/2017	Mafra, Lisboa	868	Trauma	1	-	-

Table S1: (cont.)

Wildlife Rescue Centre	Species	Entrance date	Local	Weight (g)	Probable cause of death	Body condition	Gender	Age
LxCRAS	*Larus fuscus*	Mar/11/2017	Almada, Setúbal	-	Unknown	3	Male	-
	Larus fuscus	Mar/12/2017	Almada, Setúbal	730	Biotoxins	2	Female	-
	Larus michahellis	Mar/13/2017	Manique	-	Trauma	3	-	-
	Larus fuscus	Mar/13/2017	Almada, Setúbal	-	Trauma	4	Male	-
	Larus fuscus	Mar/14/2017	Lisboa	534	Trauma	3	Male	-
	Larus fuscus	Mar/15/2017	Lisboa	771	Disease	1	-	-
	Larus fuscus	Mar/15/2017	Cascais, Lisboa	-	Run over	1	Female	-
	Larus michahellis	Mar/15/2017	Almada, Setúbal	720	Biotoxins	3	Female	-
	Larus fuscus	Mar/20/2017	Almada, Setúbal	551	Trauma and biotoxins	1	Female	-
	Larus fuscus	Feb/21/2017	Sintra, Lisboa	682	Trauma	2	-	-
	Morus bassanus	Mar/21/2017	Cascais, Lisboa	1782	Trauma	1	Male	-
	Larus fuscus	Mar/21/2017	Almada, Setúbal	720	Trauma	2	Male	-
	Larus fuscus	Mar/22/2017	Lisboa	798	Biotoxins	1	-	-

Table S1: (cont.)

Wildlife Rescue Centre	Species	Entrance date	Local	Weight (g)	Probable cause of death	Body condition	Gender	Age
LxCRAS	*Larus fuscus*	Mar/22/2017	Almada, Setúbal	762	Trauma	2	-	-
	Larus fuscus	Mar/26/2017	Lisboa	-	Run over	2	-	-
	Ciconia ciconia	Apr/01/2017	Lisboa	2792	Trauma	2	Male	-
RIAS	*Bubulcus ibis*	May/15/2014	Olhão	335	Trauma	-	-	Adult
	Ixobrychus minutus	Aug/18/2014	Olhão	105	Trauma	-	-	-
	Larus michahellis	Sept/02/2014	Quarteira	-	Unknown	-	-	Juvenile
	Egretta garzetta	Oct/10/2014	Faro	-	Weakness/ Malnutrition	-	-	-
	Larus fuscus	Oct/25/014	Almancil, Loulé	820	Disease	-	-	Adult
	Larus fuscus	Nov/18/2014	Monchique, Faro	-	Disease	-	-	-
	Bubulcus ibis	Nov/25/2014	Portimão	254	Trauma	-	-	-
	Ciconia ciconia	Mar/16/2016	Portimão	3750	Unknown	2	-	Adult
	Bubulcus ibis	Apr/03/2016	Vilamoura	320	Trauma	3	Male	-
	Larus michahellis	Jun/09/2016	Lagos	820	Trauma	2	-	-

Table S1: (cont.)

Wildlife Rescue Centre	Species	Entrance date	Local	Weight (g)	Probable cause of death	Body condition	Gender	Age
RIAS	*Larus fuscus*	Jun/15/2016	Lagoa, Portimão	570	Disease	1	Female	Sub-adult
	Larus michahellis	Jun/17/2016	Portimão	360	Fell off the nest	1	-	Chick
	Larus michahellis	Jun/22/2016	Portimão	845	Trauma	1	Male	Adult
	Larus michahellis	Jun/23/2016	Lagos	690	Trauma	1	-	Adult
	Larus michahellis	Jun/27/2016	Lagos	1015	Trapped	3	Male	Adult
	Larus michahellis	Jun/28/2016	Portimão	680	Trauma	2	Female	Adult
	Larus michahellis	Jul/01/2016	Lagos	590	Trauma	1	-	Juvenile
	Ciconia ciconia	Jul/04/2016	Olhão	3450	Fell off the nest	-	-	Juvenile
	Larus michahellis	Jul/05/2016	Lagos	500	Trauma	1	-	Chick
	Larus michahellis	Jul/05/2016	Lagos	485	Trauma	2	-	Chick
	Larus michahellis	Jul/06/2016	Olhão	770	Trapped	2	Male	Sub-adult
	Ciconia ciconia	Jul/11/2016	Olhão	-	Unknown	4	Male	Juvenile
	Larus michahellis	Jul/12/2016	Armação de Pêra, Silves	875	Trauma	2	-	Juvenile

Table S1: (cont.)

Wildlife Rescue Centre	Species	Entrance date	Local	Weight (g)	Probable cause of death	Body condition	Gender	Age
RIAS	*Larus michahellis*	Jul/12/2016	Lagos	593	Trauma	1	-	Juvenile
	Larus michahellis	Jul/15/2016	Lagos	399	Trauma	1	Male	Juvenile
	Larus michahellis	Jul/15/2016	Silves	869	Trauma	3	-	Adult
	Larus michahellis	Jul/18/2016	Albufeira	671	Trauma	4	Female	Adult
	Larus michahellis	Jul/18/2016	Albufeira	701	Trauma	4	-	Juvenile
	Larus michahellis	Jul/19/2016	Albufeira	753	Trauma	2	Female	Juvenile
	Larus michahellis	Jul/20/2016	Albufeira	873	Trauma	3	-	Juvenile
	Larus michahellis	Jul/21/2016	Portimão	705	Trapped	2	-	-
	Larus michahellis	Jul/21/2016	Portimão	887	Trauma	2	Female	Adult
	Larus michahellis	Jul/25/2016	Portimão	678	Weakness/ Malnutrition	1	-	-
	Larus michahellis	Jul/25/2016	Albufeira	620	Disease	2	-	-
	Larus michahellis	Jul/25/2016	Albufeira	870	Trauma	1	-	-
	Larus michahellis	Jul/25/2016	Quarteira	966	Disease	1	Female	Adult

Table S1: (cont.)

Wildlife Rescue Centre	Species	Entrance date	Local	Weight (g)	Probable cause of death	Body condition	Gender	Age
RIAS	*Larus michahellis*	Jul/27/2016	Portimão	-	Unknown	3	Female	Sub-adult
	Larus michahellis	Jul/27/2016	Albufeira	575	Trauma	3	Female	Adult
	Larus michahellis	Jul/28/2016	Vilamoura	100	Trauma	4	-	Juvenile
	Larus michahellis	Jul/29/2016	Olhos de Água, Albufeira	648	Trauma	2	-	-
	Larus michahellis	Jul/29/2016	Albufeira	703	Disease	4	Female	Sub-adult
	Larus michahellis	Jul/29/2016	Albufeira	-	Trauma	1	Female	Adult
	Morus bassanus	Aug/01/2016	Portimão	-	Unknown	3	Male	Adult
	Morus bassanus	Aug/02/2016	Tavira	2045	Weakness/ Malnutrition	1	-	Sub-adult
	Morus bassanus	Aug/02/2016	Loulé	1800	Trapped	2	-	Sub-adult
	Ciconia ciconia	Aug/05/2016	Portimão	3139	Electrocution	2	Female	Adult
	Larus michahellis	Aug/08/2016	Lagos	740	Trauma	1	Female	Juvenile
	Larus michahellis	Aug/12/2016	Albufeira	-	Trauma	1	-	Juvenile
	Larus michahellis	Aug/12/2016	Albufeira	740	Trauma	2	Male	-

Table S1: (cont.)

Wildlife Rescue Centre	Species	Entrance date	Local	Weight (g)	Probable cause of death	Body condition	Gender	Age
RIAS	*Morus bassanus*	Aug/12/2016	Albufeira	1741	Weakness/ Malnutrition	2	Male	-
	Larus michahellis	Aug/17/2016	Vila do Bispo	860	Trauma	3	Male	Juvenile
	Larus audouinii	Aug/17/2016	Silves	381	Trauma	3	-	Juvenile
	Larus michahellis	Aug/17/2016	Armação de Pêra, Silves	698	Trauma	2	Male	Sub-adult
	Larus michahellis	Aug/23/2016	Loulé	-	Disease	3	Male	Sub-adult
	Larus fuscus	Aug/24/2016	Almancil, Loulé	675	Trauma	2	Male	Adult
	Larus michahellis	Aug/25/2016	Vila Real de St. António	821	Trauma	3	-	-
	Larus fuscus	Aug/25/2016	Almancil, Loulé	927	Disease	4	Male	Adult
	Larus michahellis	Aug/26/2016	Olhão	-	Trauma	2	Female	Adult
	Larus michahellis	Aug/26/2016	Silves	-	Trauma	1	Female	Adult
	Larus michahellis	Aug/29/2016	Portimão	-	Trauma	2	-	Sub-adult
	Larus michahellis	Aug/29/2016	Portimão	-	Unknown	-	-	-
	Larus michahellis	Aug/31/2016	Albufeira	532	Unknown	1	Male	Juvenile

Table S1: (cont.)

Wildlife Rescue Centre	Species	Entrance date	Local	Weight (g)	Probable cause of death	Body condition	Gender	Age
RIAS	*Larus michahellis*	Aug/31/2016	Almancil, Loulé	867	Disease	2	-	-
	Larus michahellis	Aug/31/2016	Armação de Pêra, Silves	790	Disease	2	-	Adult
	Larus michahellis	Sept/01/2016	Lagoa, Portimão	-	Disease	1	Female	Adult
	Larus michahellis	Sept/02/2016	Portimão	-	Trauma	1	Female	Adult
	Larus fuscus	Sept/03/2016	Quelfes, Olhão	-	Trauma	2	Male	Adult
	Larus michahellis	Sept/03/2016	Armação de Pêra, Silves	549	Trauma	2	-	Juvenile
	Larus michahellis	Sept/06/2016	Quelfes, Olhão	822	Trapped	2	Male	-
	Larus michahellis	Sept/09/2016	Portimão	693	Disease	2	-	Sub-adult
	Larus michahellis	Sept/09/2016	Albufeira	-	Trauma	3	-	Adult
	Larus michahellis	Sept/09/2016	Faro	887	Trauma	3	-	Juvenile
	Larus michahellis	Sept/13/2016	Portimão	678	Trauma	2	-	Juvenile
	Larus michahellis	Sept/14/2016	Lagos	646	Trauma	-	-	-
	Larus michahellis	Sept/14/2016	Ilha de Faro	724	Trapped	2	Male	Juvenile

Table S1: (cont.)

Wildlife Rescue Centre	Species	Entrance date	Local	Weight (g)	Probable cause of death	Body condition	Gender	Age
RIAS	*Larus michahellis*	Sept/15/2016	Vilamoura	-	Unknown	2	Male	Juvenile
	Larus fuscus	Sept/15/2016	Tavira	-	Trauma	-	-	Juvenile
	Larus michahellis	Sept/17/2017	Vilamoura	723	Trauma	3	Female	Juvenile
	Larus fuscus	Sept/17/2017	Quarteira	725	Disease	1	Male	Juvenile
	Larus michahellis	Sept/19/2016	Vila Real de St. António	707	Trauma	2	Male	Juvenile
	Morus bassanus	Sept/22/2016	Portimão	1484	Weakness/ Malnutrition	-	-	Sub-adult
	Larus fuscus	Sept/26/2016	Faro	641	Disease	2	-	Juvenile
	Larus fuscus	Sept/27/2016	Albufeira	659	Disease	4	-	Juvenile
	Larus michahellis	Sept/27/2016	Vila Real de St. António	823	Trauma	3	-	-
	Morus bassanus	Sept/28/2016	Portimão	-	Unknown	2	Male	Juvenile
	Larus michahellis	Sept/29/2016	Quarteira	-	Disease	-	-	Juvenile
	Larus michahellis	Sept/29/2016	Quarteira	779	Disease	-	-	Juvenile
	Larus fuscus	Sept/29/2016	Portimão	708	Disease	3	Female	Juvenile

Table S1: (cont.)

Wildlife Rescue Centre	Species	Entrance date	Local	Weight (g)	Probable cause of death	Body condition	Gender	Age
RIAS	*Larus fuscus*	Sept/30/2016	Vilamoura	617	Disease	2	Female	Adult
	Morus bassanus	Sept/30/2016	Lagoa, Portimão	1552	Weakness/ Malnutrition	2	-	Juvenile
	Larus michahellis	Sept/30/2016	Quarteira	-	Unknown	4	-	Juvenile
	Larus fuscus	Sept/30/2016	Portimão	800	Disease	3	Female	Adult
	Larus michahellis	Sept/30/2016	Portimão	760	Trauma	3	Female	Adult
	Bubulcus ibis	Oct/02/2016	Almancil, Loulé	340	Trauma	2	Female	-
	Larus fuscus	Oct/02/2016	Quarteira	545	Disease	2	-	-
	Larus michahellis	Oct/02/2016	Almancil, Loulé	795	Disease	2	-	-
	Larus michahellis	Oct/03/2016	Tavira	594	Disease	2	-	-
	Larus fuscus	Oct/04/2016	Portimão	-	Unknown	3	Female	Juvenile
	Larus fuscus	Oct/04/2016	Portimão	770	Disease	1	-	Adult
	Larus fuscus	Oct/04/2016	Guia, Albufeira	692	Disease	1	-	Juvenile
	Larus fuscus	Oct/07/2016	Vilamoura	939	Disease	3	Male	Adult

Table S1: (cont.)

Wildlife Rescue Centre	Species	Entrance date	Local	Weight (g)	Probable cause of death	Body condition	Gender	Age
RIAS	*Larus fuscus*	Oct/07/2016	Almancil, Loulé	551	Unknown	-	-	-
	Larus michahellis	Oct/11/2016	Portimão	848	Trauma	1	-	Sub-adult
	Larus michahellis	Oct/13/2016	Almancil, Loulé	-	Disease	4	-	Adult
	Larus michahellis	Oct/14/2016	Portimão	805	Trauma	2	-	Adult
	Morus bassanus	Oct/18/2016	Tavira	1516	Weakness/ Malnutrition	-	-	Juvenile
	Larus fuscus	Oct/18/2016	Quarteira	604	Disease	-	-	Juvenile
	Larus fuscus	Oct/18/2016	Faro	755	Disease	-	-	Adult
	Larus fuscus	Oct/19/2016	Quarteira	-	Disease	-	-	Adult
	Larus michahellis	Oct/20/2016	Lagos	-	Trauma	-	-	Juvenile
	Larus michahellis	Oct/21/2016	Albufeira	916	Trauma	1	Male	Adult
	Larus michahellis	Oct/22/2016	Albufeira	664	Trauma	-	-	Adult
	Larus michahellis	Oct/22/2016	Alvor, Portimão	-	Unknown	-	-	Adult
	Morus bassanus	Oct/22/2016	Portimão	1504	Trauma	-	-	-

Table S1: (cont.)

Wildlife Rescue Centre	Species	Entrance date	Local	Weight (g)	Probable cause of death	Body condition	Gender	Age
RIAS	*Morus bassanus*	Oct/22/2016	Lagos	-	Unknown	-	-	-
	Morus bassanus	Oct/24/2016	Olhão	1660	Weakness/ Malnutrition	-	-	Juvenile
	Larus michahellis	Oct/26/2016	Quarteira	-	Unknown	2	Male	Adult
	Morus bassanus	Oct/26/2016	Albufeira	-	Unknown	-	-	Juvenile
	Larus fuscus	Oct/26/2016	Portimão	-	Trauma	-	-	-
	Larus fuscus	Oct/26/2016	Vilamoura	640	Disease	3	Female	Juvenile
	Larus fuscus	Oct/27/2016	Olhão	555	Unknown	3	Female	Adult
	Morus bassanus	Oct/28/2016	Castro Marim	-	Weakness/ Malnutrition	4	Male	Juvenile
	Morus bassanus	Oct/28/2016	Albufeira	-	Weakness/ Malnutrition	2	Male	Juvenile
	Larus fuscus	Oct/28/2016	Lagos	650	Trauma	2	Male	Adult
	Larus michahellis	Oct/28/2016	Portimão	540	Trauma	-	-	-
	Larus fuscus	Oct/28/2016	Vilamoura	-	Trauma	2	Male	Juvenile
	Larus michahellis	Oct/30/2016	Portimão	640	Disease	2	Female	Adult

Table S1: (cont.)

Wildlife Rescue Centre	Species	Entrance date	Local	Weight (g)	Probable cause of death	Body condition	Gender	Age
RIAS	*Larus fuscus*	Nov/02/2016	Portimão	640	Disease	3	-	Juvenile
	Morus bassanus	Nov/03/2016	Lagos	2225	Weakness/ Malnutrition	-	-	Adult
	Morus bassanus	Nov/03/2016	Albufeira	-	Trauma	-	-	Juvenile
	Larus fuscus	Nov/04/2016	Olhão	735	Trauma	-	-	-
	Larus michahellis	Nov/07/2016	Albufeira	-	Trauma	-	-	-
	Larus fuscus	Nov/07/2016	Faro	515	Disease	1	-	Sub-adult
	Larus michahellis	Nov/08/2016	Vilamoura	590	Trauma	-	-	Juvenile
	Larus fuscus	Nov/10/2016	Carvoeiro, Lagoa	625	Weakness/ Malnutrition	1	-	Sub-adult
	Larus fuscus	Nov/14/2016	Albufeira	510	Trauma	-	-	-
	Ardea cinerea	Nov/18/2016	Olhão	1170	Trauma	4	Female	Adult
	Larus fuscus	Nov/18/2016	Castro Marim	510	Trauma	-	-	Adult
	Larus fuscus	Nov/21/2016	Olhão	690	Trauma	-	-	-
	Larus michahellis	Nov/21/2016	Albufeira	-	Trauma	-	-	Juvenile

Table S1: (cont.)

Wildlife Rescue Centre	Species	Entrance date	Local	Weight (g)	Probable cause of death	Body condition	Gender	Age
RIAS	*Larus michahellis*	Nov/21/2016	Portimão	570	Trauma	1	Female	Juvenile
	Larus michahellis	Nov/22/2016	Lagoa	630	Trauma	1	Female	Adult
	Larus melanocephalus	Nov/23/2016	Portimão	290	Disease	2	Female	Sub-adult
	Ardea cinerea	Nov/23/2016	Silves	975	Gunshot	1	Male	Adult
	Larus fuscus	Nov/26/2016	Olhão	750	Trauma	3	Female	Adult
	Larus michahellis	Dec/02/2016	Ilha de Faro	-	Trauma	-	-	Juvenile
	Larus fuscus	Dec/02/2016	Faro	-	Run over	2	Female	-
	Larus ridibundus	Dec/06/2016	Olhão	215	Trauma	-	-	Sub-adult
	Larus fuscus	Dec/06/2016	Albufeira	-	Unknown	-	-	Sub-adult
	Larus fuscus	Dec/09/2016	Olhão	755	Trauma	-	-	Juvenile
	Larus fuscus	Dec/11/2016	Portimão	-	Unknown	-	-	Adult
	Larus fuscus	Dec/11/2016	Olhão	750	Disease	2	Male	Juvenile
	Larus michahellis	Dec/14/2016	Quarteira	780	Disease	3	Female	Juvenile

Table S1: (cont.)

Wildlife Rescue Centre	Species	Entrance date	Local	Weight (g)	Probable cause of death	Body condition	Gender	Age
RIAS	*Larus fuscus*	Dec/15/2016	Tavira	-	Run over	3	Female	Adult
	Larus michahellis	Dec/19/2016	Faro	-	Disease	4	-	Adult
	Larus michahellis	Dec/20/2016	Portimão	927	Trauma	3	Male	Juvenile
	Larus michahellis	Dec/21/2016	Albufeira	643	Trauma	2	Male	Juvenile
	Larus fuscus	Dec/22/2016	Alvor, Portimão	577	Disease	1	-	Adult
	Larus michahellis	Dec/23/2016	Albufeira	968	Disease	4	Female	Juvenile
	Larus fuscus	Dec/27/2016	Portimão	681	Disease	-	-	Adult
	Larus fuscus	Dec/27/2016	Moncarapacho, Olhão	606	Trauma	2	-	Sub-adult
	Larus michahellis	Dec/29/2016	Portimão	772	Disease	3	Male	Adult
	Larus fuscus	Jan/02/2017	Quarteira	-	Trauma	4	Male	Adult
	Larus fuscus	Jan/04/2017	Quarteira	778	Disease	4	-	Adult
	Larus ridibundus	Jan/05/2017	Loulé	225	Disease	-	-	Sub-adult
	Larus fuscus	Jan/07/2017	Altura, Castro Marim	500	Trauma	3	Female	Juvenile

Table S1: (cont.)

Wildlife Rescue Centre	Species	Entrance date	Local	Weight (g)	Probable cause of death	Body condition	Gender	Age
RIAS	*Larus michahellis*	Jan/10/2017	Portimão	725	Disease	1	Male	Adult
	Larus michahellis	Jan/11/2017	Carvoeiro, Lagoa	780	Trauma	2	Female	Adult
	Larus fuscus	Jan/11/2017	Faro	775	Disease	3	Male	Juvenile
	Larus fuscus	Jan/19/2017	Vila Real de St. António	562	Trauma	-	-	Adult
	Larus michahellis	Jan/19/2017	Carvoeiro, Lagoa	940	Trauma	3	Female	Adult
	Larus michahellis	Jan/19/2017	Armação de Pêra, Silves	747	Trauma	4	Female	Adult
	Larus fuscus	Jan/19/2017	Quarteira	-	Unknown	2	Male	Juvenile
	Larus michahellis	Jan/23/2017	Silves	682	Trauma	2	Female	Juvenile
	Ardea cinerea	Jan/27/2017	Almancil, Loulé	-	Unknown	-	-	Adult
	Larus michahellis	Jan/30/2017	Lagos	-	Trapped	1	Male	Adult
	Larus michahellis	Feb/03/2017	Silves	598	Trauma	2	Female	Adult
	Larus fuscus	Feb/03/2017	Olhão	-	Trauma	4	-	Adult
	Ciconia ciconia	Feb/03/2017	Beja	-	Trauma	3	Female	-

Table S1: (cont.)

Wildlife Rescue Centre	Species	Entrance date	Local	Weight (g)	Probable cause of death	Body condition	Gender	Age
RIAS	*Larus michahellis*	Feb/09/2017	Armação de Pêra, Silves	879	Trauma	4	-	Adult
	Larus fuscus	Feb/14/2017	Portimão	873	Disease	4	Male	Adult
	Larus fuscus	Feb/15/2017	Vilamoura	721	Trauma	2	Male	Juvenile
	Larus fuscus	Feb/15/2017	Silves	455	Trauma	1	Male	Adult
	Larus fuscus	Feb/16/2017	Vilamoura	717	Trauma	2	-	Sub-adult
	Larus fuscus	Feb/18/2017	Portimão	730	Disease	2	Female	Adult
	Larus fuscus	Feb/18/2017	Vilamoura	771	Trauma	3	-	Juvenile
	Larus fuscus	Feb/27/2017	Olhão	628	Disease	-	-	Adult
	Larus fuscus	Feb/27/2017	Portimão	-	Unknown	2	Female	Adult
	Larus fuscus	Feb/27/2017	Portimão	-	Unknown	1	Male	Adult
	Larus fuscus	Feb/28/2017	Portimão	-	Unknown	5	Female	Adult
	Larus fuscus	Feb/28/2017	Portimão	812	Disease	4	Male	Adult
	Larus fuscus	Feb/28/2017	Portimão	919	Disease	4	Male	Adult

Table S1: (cont.)

Wildlife Rescue Centre	Species	Entrance date	Local	Weight (g)	Probable cause of death	Body condition	Gender	Age
RIAS	*Larus fuscus*	Mar/04/2017	Quarteira	654	Disease	1	Male	Adult
	Larus fuscus	Mar/05/2017	Olhão	912	Collision with a structure	3	Male	Juvenile
	Larus fuscus	Mar/11/2017	Albufeira	743	Disease	3	Male	Juvenile
	Ciconia ciconia	Mar/14/2017	Castro Verde, Beja	3131	Trauma	-	Male	Adult
	Larus fuscus	Mar/15/2017	Quarteira	-	Disease	4	Male	Sub-adult
	Larus michahellis	Mar/17/2017	Portimão	-	Unknown	4	Male	Adult
	Larus fuscus	Mar/17/2017	Portimão	857	Disease	2	Male	Adult
	Ciconia ciconia	Mar/23/2017	Almodôvar, Beja	3387	Unknown	4	Male	Adult
	Larus ridibundus	Mar/26/2017	Almancil, Loulé	-	Unknown	2	Female	Adult
	Ciconia ciconia	Mar/27/2017	Mértola, Beja	3177	Trauma	3	Female	Adult
	Ciconia ciconia	Apr/08/2017	Olhão	-	Trauma	2	Female	Adult
	Ciconia ciconia	May/10/2017	Moura, Beja	-	Trauma	3	Male	Adult
	Morus bassanus	May/25/2017	Fuseta, Olhão	-	Trauma	-	-	Juvenile

Table S1: (cont.)

Wildlife Rescue Centre	Species	Entrance date	Local	Weight (g)	Probable cause of death	Body condition	Gender	Age
RIAS	*Morus bassanus*	May/29/2017	Montenegro, Faro	1760	Trauma	3	Male	Adult

Table S 2: Sample description of dataset A. Male (M) or female (F), chick (C), juvenile (J), sub-adult (S-A) or adult (A). Note that gender and/or age could not always be determined.

Species	Sample size (n)	Gender		Age			
		M	F	C	J	S-A	A
Larus michahellis	124	29	41	3	42	9	46
Larus fuscus	107	31	22	0	22	7	34
Ciconia ciconia	58	22	17	12	15	0	29
Morus bassanus	21	9	0	0	9	4	4
Ardea cinerea	17	8	4	0	7	0	7
Bubulcus ibis	4	1	1	0	0	0	1
Larus ridibundus	4	0	2	0	0	2	1
Melanitta nigra	2	-	-	0	2	0	0
Alca torda	1	-	-	-	-	-	-
Ciconia nigra	1	-	-	0	1	0	0
Egretta garzetta	1	-	-	-	-	-	-
Gavia stellata	1	-	-	0	1	0	0
Ixobrychus minutus	1	-	-	-	-	-	-
Larus argentatus	1	1	0	0	0	1	0
Larus audouinii	1	-	-	0	1	0	0
Larus melanocephalus	1	0	1	0	0	1	0
Phalacrocorax carbo	1	-	-	0	0	0	1
Platalea leucorodia	1	1	0	0	1	0	0
Rissa tridactyla	1	0	1	0	0	0	1

Table S 3: Sample description of dataset B. Male (M) or female (F), chick (C), juvenile (J), sub-adult (S-A) or adult (A). Note that gender and/or age could not always be determined.

Species	Sample size (n)	Gender		Age			
		M	F	C	J	S-A	A
Larus michahellis	96	21	26	3	33	8	35
Larus fuscus	68	22	14	0	20	7	33
Morus bassanus	18	6	0	0	9	3	3
Ciconia ciconia	10	4	4	0	2	0	7
Bubulcus ibis	4	1	1	0	0	0	1
Ardea cinerea	3	1	1	0	0	0	3
Larus ridibundus	3	0	1	0	0	2	1
Egretta garzetta	1	-	-	-	-	-	-
Ixobrychus minutus	1	-	-	-	-	-	-
Larus audouinii	1	-	-	-	1	-	-
Larus melanocephalus	1	0	1	0	0	1	0

Table S 4: Sample description of dataset C. Male (M) or female (F), chick (C), juvenile (J), sub-adult (S-A) or adult (A). Note that gender and/or age could not always be determined.

Species	Region	Sample size (n)	Gender		Age			
			M	F	C	J	S-A	A
Ciconia ciconia	North	47	17	13	12	13	0	22
	South	11	5	4	0	2	0	7