A SIX-YEAR SURVEY OF PLASTIC INGESTION BY AQUATIC BIRDS IN SOUTHERN PORTUGAL

Silvia Rao[1], Katy R. Nicastro[1,2,3], María Casero[4], Christopher D. McQuaid[2], Gerardo I. Zardi[2]*

[1]CCMAR-CIMAR – Associated Laboratory, University of Algarve, Campus de Gambelas, Faro 8005–139, Portugal
[2]Department of Zoology and Entomology, Rhodes University, Grahamstown 6140, South Africa
[3]Univ. Lille, CNRS, Univ. Littoral Côte d'Opale, UMR 8187, LOG, Laboratoire d'Océanologie et de Géosciences, Wimereux, France
[4]Wildlife Rehabilitation and Investigation Centre (RIAS) – Associação ALDEIA, Ria Formosa Natural Park, Olhão, Portugal

*Corresponding Author: Email: zardi73@yahoo.it

ABSTRACT

Anthropogenic litter in the environment is pervasive globally. Of particular concern are plastics because of their ubiquity, longevity in the environment and lethal effects. Plastics affect organisms at most levels of biological organization but, even in well studied animals like birds, we have limited insight into species-specific vulnerability or temporal trends of ingestion. We examined stomach contents of four aquatic bird species over more than six years in southern Portugal. Of the 462 individuals analysed, 22.7% had ingested anthropogenic litter, predominantly plastics, most of which were clear or white in colour. The White Stork, *Ciconia ciconia*, exhibited the highest frequency of occurrence of anthropogenic litter (61.1%) and of plastics specifically (55.6%), while the Yellow-legged Gull, *Larus michahellis*, had the lowest (13.4% for both anthropogenic litter and plastics). Similar frequencies of occurrence were found for the Northern Gannet, *Morus bassanus* (22% and 20.3%) and the Lesser Black-backed Gull, *Larus fuscus* (21.4% and 20.8%). The composition of the plastic ingested varied throughout the study period for each species, but with no clear temporal pattern. Our results revealed the ubiquity and frequency of occurrence of plastic ingestion as well as clear differences among species, providing a basis for long-term monitoring of litter ingestion.

Keywords: multispecies, plastic debris, environmental monitoring, temporal trend, coastal lagoon

INTRODUCTION

Over the last 70 years, the abundance of anthropogenic litter, in particular that made of plastic, has increased exponentially and has rapidly emerged as a global threat to biodiversity (Lau et al., 2020). The rapid increase and significant accumulation of plastics has been particularly pronounced in marine habitats (Haward, 2018). Over recent years, scientific literature has highlighted the ubiquitous nature of plastic pollution from coastlines to the open ocean, and from the sea surface to the seafloor (Almroth and Eggert, 2019). Beyond the aesthetic effects of plastic litter, mounting evidence shows that the range of taxa affected by this long-lasting material and the potentially harmful consequences have escalated (Jâms et al., 2020).

Aquatic birds are especially susceptible to the pervasive and increasing presence of plastic materials in the environment because of their high trophic level and extensive foraging ranges (Avery-Gomm et al., 2012). Some of the earliest indications of plastic pollution in marine organisms were plastic caps, toys and bags ingested by Laysan albatrosses in the 1960s (Kenyon and Kridler, 1969). Since then, the number of studies stressing the wide range of deleterious effects of plastic pollution on aquatic birds has increased enormously (Battisti et al., 2019). The number of avian species negatively affected by anthropogenic waste is projected to grow as more species are investigated; predictably, by 2050, 99% of all seabirds species might have ingested plastic (Wilcox et al., 2015).

Anthropogenic pollution has a wide range of negative effects on aquatic birds, mainly through ingestion and entanglement (e.g. Gall and Thompson, 2015; O'Hanlon et al., 2017; Provencher et al., 2017). Entanglement is mostly passive, when individuals become trapped in litter such as fishing nets or plastic bags, but entanglement can also occur when individuals became trapped in anthropogenic items that they collect purposely, for example, to construct their nests (Ryan, 2018; Lopes et al., 2020). Ingestion can occur inadvertently, while foraging on other prey items, or deliberately when anthropogenic materials resemble natural food items (Cadée, 2002; Wilcox et al., 2015, Lopes at al. 2021). Litter kept within the gut can have direct effects such as dietary dilution causing impaired feeding and growth (Ryan, 1988), and physical damage as internal wounds and ulcers, and gastrointestinal obstruction (Puskic et al., 2020). Further, a growing body of evidence shows that birds carrying plastics in their stomachs are exposed to potential toxicological effects arising from leaching of contaminants that were either added during plastic production (e.g. flame retardant chemicals) or absorbed by the plastic's surface from the surrounding environment (Roman et al., 2019; Guo et al., 2020; Tamara et al. 2020).

Litter ingested by birds does not necessarily mirror the abundance of plastic waste or anthropogenic material in the environment. However, plastic waste is a reliable proxy for spatial and temporal trends in the abundance and typology of plastic litter in the environment (e.g., Van Franeker et al., 2011; Van Franeker and Law, 2015). For instance, the Northern Fulmar *Fulmarus glacialis*, a procellariiform seabird distributed across the North Atlantic and Pacific Ocean (Mallory et al., 2006), is used by both OSPAR (Oslo/Paris Convention for the Protection of the Marine Environment of the North-East Atlantic) and the European MSFD (Marine Strategy Framework Directive) to monitor spatio-temporal fluctuations of plastic waste in the North Sea (OSPAR Commission, 2008). The identification and monitoring of such sentinel species is important to the investigation of plastic pollution (Mallory et al., 2006; Provencher et al., 2009; Van Franeker et al., 2011; Avery-Gomm et al., 2012), but narrows the focus. The monitoring of multiple species, including non-indicator species, provides a broader understanding of the ubiquity of plastic ingestion and the factors that render different species more or less vulnerable to its ill effects. This allows the recognition of how vulnerability is influenced by the qualities of the many types of plastics (e.g. their colour), as well as the characteristics of the monitored species as temporal trends of plastic ingestion differ vastly among species and plastic typologies (Van Franeker et al., 2011). For instance, surface-feeding seabirds tend to ingest more plastic than pursuit-diving birds because the majority of plastics float and accumulate at the surface (O'Hanlon et al., 2017; Poon et al., 2017). Over the last few years, several multi-species investigations have been crucial to acquire a wide-ranging picture of marine ecosystem health and have highlighted the value of using multiple species in monitoring programmes (e.g. Acampora et al., 2016).

Southern Portugal is characterized by several coastal lagoons, some of which are areas of high wildlife diversity and act as key migration stopover and breeding sites for over 100 bird species. Recently, baseline assessments of the frequency of occurrence of plastic litter and its effects on multiple species of aquatic birds in southern Portugal have shown that the abundance and types of ingested litter vary considerably among species (Nicastro et al., 2018; Basto et al., 2019). Here, we used data from these baseline studies in combination with more recent data from individuals which entered a wildlife recovery centre (RIAS) to assess temporal changes in litter ingestion by multiple species of aquatic birds in southern Portugal. Specifically, we examined the ingestion of litter by White Stork *Ciconia ciconia*, Lesser Black-backed *Larus fuscus* and Yellow-legged Gulls *Larus michahellis* and Northern Gannet *Morus bassanus* over a period of nearly 7 years (2014 to mid 2020). Importantly, all birds considered in this study are from the recovery centre RIAS, thus minimising the biases that often occur when comparing plastic ingestion by rescued birds and conspecifics from the wild (Provencher et al. 2019).

With plastic pollution increasing visibility, we expected rising frequency of plastic ingestion over the 7 years period. Further, because the species targeted in this study vary greatly in their foraging range and diet, we hypothesised pronounced interspecific differences in plastic ingestion. Specifically, because plastic contamination is expected to be higher in offshore waters compared to nearshore waters (Avery-Gomm et al. 2013 and references therein), we hypothesised that plastic ingestion would be higher in species that mainly forage offshore than in species that are predominantly reliant on coastal habitats for food.

MATERIAL AND METHODS

Ethic statement

Animals that reached the center had diseases or were injured. Birds were dead on arrival or euthanised after 24 hours when they showed no signs of recovery. No animals were euthanised for the benefit of the project. All applicable institutional, national and international guidelines for the care and use of animals were followed. Animal manipulation in the RIAS was performed by suitably qualified professionals, according to the directive 86/609/EEC. All experimental assays, with or without the use of animals were performed under Government Veterinary Service (Direcao Geral de Veterinaria/DGV).

Study species

The White Stork (*Ciconia ciconia*) is globally rated as a species of least concern (IUCN, 2018); its distributional range spans across Europe, the Middle East, North and South Africa (Cramp and Simmons, 1977). In Europe, two populations occur with distinct migratory routes and wintering areas. The occidental population mainly migrates over the Strait of Gibraltar, while the oriental population mainly crosses over the Bosphorus and Israel (Araújo et al., 1998). In Portugal, most of the population breeds in the south (Araújo et al., 1998). The White Stork primarily feeds on insects, larvae, amphibian, reptiles, small mammals, annelids and aquatic organisms (Cheriak et al., 2014; Tsachalidis & Goutner, 2002; Vrezec, 2009).

The Lesser Black-backed Gull (*Larus fuscus*) is classified as a less vulnerable species, however, a population reduction over the years has been observed (IUCN, 2018). It is a palearctic bird but is mostly distributed in the United Kingdom (Hagemeijer et al., 1997). In the Iberian Peninsula, this species is commonly found on the Berlengas Archipelago, on the Pessegueiro Island and in the Ria Formosa coastal lagoon. In Portugal, it nests in estuaries and coastal lagoons, and it is frequently seen on Portugal´s coasts during winter. This species mainly feeds on insects, fish and human rubbish (Coulson & Coulson, 2010; Gyimesi et al., 2016; Schwemmer & Garthe, 2005; "Aves — ICNF," 2020)).

The Yellow-legged Gull (*Larus michahellis*) is listed as a species of least concern (IUCN, 2018); its distribution includes the Macaronesia Islands and Northwest Africa through the Mediterranean. It is a migratory species, but some populations are defined as partially migratory. The European population is estimated to be high and expanding in France and the

Iberian Peninsula. The species inhabits coastal as well as inland areas. The yellow-legged gull feeds on fish, insects, molluscs, small mammals and dump rubbish (“Aves — ICNF”, 2020; Calado et al. 2021).

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

Study area

The study was carried out in the south of Portugal around the lagoon system of Ria Formosa, which has recently been declared Ria Formosa Natural Park (PNRF; Amaral, 2009). The Ria Formosa system comprises a total area of about 18400 ha, of which about 3600 ha are permanently submerged. It incorporates a wide range of habitats, including marshes, sandbanks and mudflats, dunes, salt flats, lagoons and areas of diverse vegetation. The system is known for its high diversity of Avifauna (Amaral, 2009) with over one hundred species, mainly in the orders Gaviiformes, Podicipediformes, Anseriformes, Gruiformes and Charadriiformes (Farinha and Costa, 1999). These wetlands act as overwintering zones for northern species, and the southerly migration routes of many birds pass over this lagoon.

Procedure

Data collected for this study (n = 117 individual birds) were integrated with data from Nicastro et al. (2018; n = 153) and Basto et al. (2019; n = 192). Samples from these previous studies were from the same region and treated using the same procedures, described below. The combined dataset comprised 462 dissected individuals collected over six and a half years (June 2014 to May 2020) and comprised of selected aquatic and marine birds that had been brought to the wildlife recovery center RIAS, situated in Olhão, southern Portugal. Sampling was based on volunteers and therefore was irregular over time and species. The 462 individuals comprised 53 White Stork (*C. ciconia*), 154 Lesser Black-backed (*L. fuscus*) and 196 Yellow-legged Gull (*L. michahellis*) and 59 Northern Gannet (*M. bassanus*).

Necropsies were done immediately after death or samples were frozen at -20°C for later dissections and followed the Van Franeker dissection method (Provencher et al., 2019). For all samples, the gastrointestinal tract (GIT) was complete upon examination. Specifically, we assessed plastic retention within the gizzard and proventriculus. Both compartments were flushed with water while manipulating the GIT to ensure that all cracks and crevices were carefully washed; this approach is key to assess the presence of any embedded litter in the wall or lining of the GIT (Seif et al. 2018). If available, data on age class (New-Born, Juvenile, Sub-Adult or Adult), gender, body condition and cause of death were registered for each individual. Age and gender were determined by the stage of the sexual organs, the body condition (0 to 4) was defined by using pre-determined dimensions (Pinilla, 2000). The stomach contents were sieved through a 1mm mesh. Inorganic particles were collected and air-dried for two to three days in Petri dishes.

Inorganic particles were counted individually and weighed to the nearest 0.0001g (Sartorius Advantage AW-224 scale). These items were classified as litter and further divided into non-plastic items and all plastics. All plastics was further classified into the categories, industrial plastics, or user plastics. Items classified as user plastics were additionally placed in subcategories based on type of plastics or colour. Types of plastics were sheetlike, fragment, threadlike, foamed, others (Van Franeker et al., 2011) while colours were white/clear, grey-silver, black, blue-purple, green, orange-brown, red-pink and yellow (Provencher et al., 2017).

Data Analysis

To avoid unbalanced sampling, years were grouped in three-year intervals (2014-2015, 2016-2017 and 2018-2020). Additionally, since only three birds ingested industrial plastics, this category was not included in the analyses to avoid highly zero-inflated data.

Initially, data on user plastic ingested were analysed for the effect of species and year interval in a two-way ANOVA. However, the assumption for homogeneity of variances (Levene's test) were not fulfilled for either total counts or mass of ingested plastics, even after transformation

(Sqrt or Log10). Thus, each species was analysed separately using a 1-way ANOVA with Year Interval as fixed factor (3 levels, 2014-2015, 2016-2017 and 2018-2020) and counts or mass as the dependent variable.

Additionally, data were tested for differences in patterns of plastic ingestion among Year Intervals. To do so, three datasets were used: (1) one with plastic counts for each category (i.e., sheetlike, fragment, threadlike, foamed, others), (2) one with plastic mass for each category, and (3) one with plastic counts for each colour category (white/clear, grey-silver, black, blue-purple, green, orange-brown, red-pink and yellow.

For each dataset, a two-way multivariate PERMANOVA test was performed with Year Intervals (3 levels; 2014-2015, 2016-2017 and 2018-2020) and species (4 levels) as fixed factors and either counts (for datasets (1) and (3)) or mass (for dataset (2)) as the dependent variable. Each analysis was run with 9999 permutations and using Bray–Curtis dissimilarity matrices with a dummy variable of 1 (Clarke et al., 2006) for square-root transformed multivariate measures. The Monte Carlo P-values (p(MC)) were preferred over the permutation P-values (Anderson, 2006).

When main effects were significant, pairwise tests were performed (Monte Carlo) and permutational analysis of multivariate dispersions test for heterogeneity (PERMDISP) was run to evaluate the variability among years, based on the distances to centroids (Anderson, 2006). In addition, the SIMPER procedure (Clarke, 1993) was used to identify the percentage contribution (%) that each variable made to the between-years Bray-Curtis dissimilarities and was run with a cut of 50%. All multivariate analyses were performed using PRIMER 6.1.15 and PERMANOVA+ 1.0.5 software (PRIMER-E Ltd, 2012).

To visually represent each dataset, a two-dimensional non-metric multidimensional scaling (nMDS) was performed highlighting the two main factors in the PETMANOVA analyses (Year, Species).

RESULTS

Of the 462 individuals, 105 ingested litter (i.e., non-plastics and all plastics; 22.73%). Of these 100 (21.65%) ingested plastics, making all plastics the dominant ingested material (Figure 1). Among the birds ingesting plastics (all plastics), industrial plastics (as opposed to user plastics) were ingested by only three individuals in the 2016-2017 period, having ingested 90, 1 or 2 pellets (Table 1) and thus this category was removed from the multivariate analyses to avoid highly zero-inflated data.

No species showed significant temporal changes in ingestion of user plastics, i.e., no significant effects of Year Interval (*L. fuscus*: counts F(2,151)=0.33, p = 0.721; mass F(2,151)=0.26, p = 0.772; *L. michahellis:* counts F(2,151)=0.65, p = 0.521, mass F(2,191)=0.108, p = 0.898; *C. ciconia:* counts: F(2, 51)=1.704, p = 0.192; mass: F(2, 51)=0.82, p = 0.445; *M. bassanus:* counts: F(2, 56)=0.78, p = 0.462; mass: F(2, 56)=0.43, p = 0.655; Figure 2g,h).

The pattern in the counts of ingested user plastics changed among Year Intervals (PERMANOVA, Year Interval, p(MC) = 0.001) and differed among species (PERMANOVA, Species, p(MC) = 0.001; Figure 3). In particular, the period 2014-2015 was significantly different from the other time frames (pair wise tests, p<0.05) while the pattern of ingestion by *C. ciconia* significantly differed from that of the other species. However, the dispersion of the datapoints within the term species was not homogenous (PERMDISP, p = 0.001) which might have inflated the effect of species.

The plastic category fragments contributed most to the differences observed between 2014-2015 and 2016-2017 (SIMPER: 38.4%) and between 2014-2015 and 2019-2020 (SIMPER: 29.5%) followed by sheetlike (SIMPER: 30.4%) and other (SIMPER: 24.4%) respectively. The categories other and threadlike contributed equally (SIMPER: 27.9% and 27.8 respectively) to the dissimilarities between 2016-2017 and 2018-2020.

The pattern in the mass of ingested user plastics changed throughout the years but not in the same way for all species (PERMANOVA, Species x Year Interval, p = 0.001; Figure 4).

However, the dispersion of the datapoints within the term Species and within the term Year Interval was not homogenous (PERMDISP, p = 0.001 for both) which may have inflated the significant effects. This is probably the case for the effect of Year Interval, as no significant differences were further detected for the term Species x Year Interval for pairs of the levels of the factor Year Interval (pair-wise; p > 0.075). However, pair-wise testing further confirmed a species effect, with plastic ingestion patterns for *C. ciconia* differing significantly from each of the other species (pair-wise; p(MC) < 0.01 for all) for every year interval.

Other and fragments contributed the most to the differences observed between *C. ciconia* and *L. fuscus* (SIMPER: 46.9 and 32.37% respectively), *L. michahellis* (47.2 and 36% respectively) and *M. bassanus* (43.6 and 26.2% respectively).

DISCUSSION

All four aquatic bird species reported here ingested anthropogenic litter, with plastic as the dominant material. Interestingly, contrary to our initial expectations and despite marked temporal fluctuations in the percentage of individuals ingesting litter, none of the species showed significant temporal trends through the study period. Similarly, despite the counts and the mass of some specific types of plastics ingested changing significantly among years, no significant trends of increasing or decreasing frequency of plastic ingestion were observed during our study.

Importantly, all the birds used in this study were either dead when they were admitted to the recovery facility or died during their stay. While the use of birds from rescue centres is an extremely valuable and widely used approach to investigate plastic ingestion, it is also critical to note that such samples may or may not be representative of the population and, thus, may affect how the data should be interpreted. In particular, the assessment of plastic ingestion may be biased in stranded birds or those for which the cause of death in unknown, as litter may have caused the death of the animal (Auman et al. 1998) or the poor health condition that eventually led to its admission to a rescue facility. Indeed, there is evidence that in some species, the plastic burdens differ significantly between beached individuals and those that died in good conditions, such as drowning in fishing nets or colliding with ship cables (Rodríguez et al. 2018).

Although numerous studies have reported anthropogenic litter ingestion in birds, temporal comparisons remain relatively scarce and have provided contrasting patterns depending on the region and the species assessed. For instance, results from a 14 year (1975-1989) survey of plastic ingestion by northwestern Atlantic seabirds showed a significant increase in Procellariiform species (Moser and Lee, 1992). In contrast, the evaluation of ingestion of plastic marine debris by Common and Thick-billed Murres in the northwestern Atlantic from 1985 to 2012 did not show a significant change in the frequency of ingestion among species or periods (Bond et al., 2013). More recent studies, looking at species-specific and temporal differences in plastic ingestion over ten years (2008 and 2018) by four seabird species in the Canadian Arctic, found that the number and mass of plastic ingested by fulmars (*Fulmarus glacialis*) decreased but the frequency of occurrence of plastic ingestion did not change (Baak et al., 2020). Despite non-significant temporal tendencies, we observed pronounced interspecies differences in plastic ingestion, confirming previous studies showing that the propensity of a species to ingest plastic often varies according to foraging behaviour, foraging range, morphology or diet (e.g. Moser and Lee, 1992; Richardson et al., 2013; Bond et al., 2014; Poon et al., 2017;). Moreover, for all the species and throughout most of the period targeted in our study, inter-individual variability was large, possibly triggered by the numerous factors affecting ingestion.

In general, because litter occurs at higher densities in offshore waters, seabirds that feed farther offshore are expected to be more prone to plastic ingestion than inshore species (Richardson et al., 2013). However, our initial hypothesis was not supported; the White Stork *Ciconia ciconia*, which generally feeds inland or in coastal shallow waters, displayed consistently higher proportions of individuals ingesting plastic than the other three species. Our samples of *C. ciconia* showed proportions of anthropogenic litter (plastic and non-plastic) ingestion comparable to values reported in other studies in Spain (e.g. Peris, 2003). However, when considering percentages of plastic ingestion alone, our values were markedly higher than those of Spanish storks (14%; Peris, 2003). Such marked differences are most likely due to distinct feeding grounds. Specifically, all samples used in Peris (2003) came from the Spanish provinces of Avila, Salamanca and Zamora (41° 30′- 40° 50′ N/6°15′-5°20′ W), situated inland on the west-central northern plateau. This plateau lies between 800- 1000 metres above sea level and it is largely devoted to cereal croplands, open grasslands, and wooded pasturelands. Critically, the area has a widespread supply of waste dumps that are exploited by storks throughout the year. Our study area, the Ria Formosa Natural Park, sustains a variety of diverse habitats, including sand dunes, saltmarshes, and foundation species such as seagrasses, and is used for various human activities. Recent studies show that the Ria Formosa Natural Park is experiencing increasing contamination by anthropogenic litter largely made up of plastics and construction materials and mainly triggered by local activities, such as tourism and fishery, as well as shipping and industry (Velez et al., 2019, 2020). Mounting evidence shows that the Ciconiidae are increasingly reliant on environments linked to human activities for food. While we cannot establish if the majority of ingested litter found in the stomachs of storks derived from human-related environments, an increasing number of studies have stressed the growing use of agricultural areas and landfill sites by European white storks with substantial behavioural and fitness alterations (Gilbert et al., 2016). For example, rubbish dumps provide nearly 70% of food for Spanish storks, the continuous availability of foraging resources at landfills compensating for seasonal declines in natural food accessibility (Tortosa et al., 2002). As a consequence, significant improvement in breeding success has been reported, largely due to reduced mortality in first-year birds (Gilbert et al., 2016). Similarly, recent studies investigating *C. ciconia* breeding colonies that exploit landfill sites in northern Algeria, have described increases in egg volume and hatching mass (Djerdali et al., 2016). The year-round availability of abundant food from rubbish dumps has also modified the migratory strategies of white storks, eventually aiding the establishment of overwintering populations in a formerly migratory species (Blanco, 1996; Tortosa et al., 2002). Most significantly, changes in population demography have also been highlighted; over the three decades, the breeding population of *C. ciconia* has increased significantly in Iberia (Rosa et al., 2009).

Several gull species are also particularly prone to litter ingestion (Lopes et al. 2021). In addition to foraging in marine habitats, they exploit land-based sites including general public litter, harbours and landfills and dumps in the proximity of the coast (Belant et al., 1998; Duhem et al., 2003; Lindborg et al., 2012; Seif et al., 2018). In fact, it has been shown that some gulls may specialise on landfills totally abandoning marine habitats for feeding (Weiser and Powell, 2011; Bond, 2016). Despite the increasing dependence on human-related environments, the Lesser Black-backed Gull *Larus fuscus* and the Yellow-legged Gull *Larus michahellis* samples assessed in our study showed consistently lower proportions of ingestion than storks which also significantly rely on land-fills for feeding. Furthermore, the two seagull species showed pronounced differences in the percentages of individuals ingesting litter, with *L. fuscus* having a noticeably higher proportions for both plastic and non-plastic items.

Previous studies along the shores of Ireland, found that all *L. fuscus* ingested plastic (Acampora et al., 2016, though only two samples were investigated). Other *Larus spp.* assessed by Acampora et al. (2016) exhibited comparable percentages of individuals ingesting litter, ranging from 22% to 32%. Previous assessments of plastic debris ingestion in the Yellow-legged Gull along Mediterranean shores (Codina-García et al., 2013) found percentages of ingestion nearly three times as high as those in our Portuguese samples.

For the Northern Gannet *Morus bassanus* the proportion of individuals ingesting anthropogenic litter was markedly lower than in *C. ciconia*. Previous assessments of plastic ingestion in the Northern Gannet from Portugal reported a relatively low proportion (4.8%; Basto et al. 2019). However, our results highlight percentages of ingestion comparable to those of Suliformes from Ireland (26.7%; Acampora et al., 2016) and similar to the 23.9% reported for Pelecaniformes by Kühn et al., 2015, and higher than values for Northern Gannet in the Mediterranean (13%; Codina-García et al., 2013). The discrepancy is probably due to the increase in sample size in this study (n= 59) compared to the previous assessment which was based on data from just 8 individuals (Basto et al. 2019).

The retention times of anthropogenic litter in a bird's digestive tract may also be a key determinant of both marked interspecific differences and spatio-temporal variations within the same species. For example, gulls regurgitate significant amounts of the debris ingested, so that retention times are likely to be markedly shorter than for other species that accumulate litter in the gut and the stomach contents of gulls will represent only a snapshot of ingestion. As for gulls, abundant evidence shows that storks are also able to regurgitate, either to deliver food onto the bottom of the nest or to expel non-edible items initially mistakenly identified as food (e.g., Sazima et al. 2015; Henry et al. 2011; Kwieciński et al. 2006). However, because retention time is also affected by qualities of the litter itself such as the morphology and material/polymer type of ingested items, storks may not be able to regurgitate as efficiently as gulls. Several studies have provided evidence of massive ingestion of elongated soft plastics (i.e., silicon rubber bands and elastics) by storks, presumably because their colour and shape mimic prey such as Lumbricidae (e.g., Sazima et al. 2015; Henry et al. 2011). Although receiving little attention, retention time of different types of plastic may be a crucial variable when assessing plastic ingestion in marine wildlife. In fact, there is evidence that microplastics have significantly shorter retention times than larger pieces, that must be broken down before being expelled (Bergmann et al., 2015) and that softer plastics such as latex balloon fragments, bags and foam can remain in the guts of marine animals for several months (Roman et al., 2019).

Supporting previous studies, we found high frequency of occurrence of User plastics ingestion. This may reflect the ease with which such material enters the environment often directly from landfills (UNEP, 2016). Currently, one-third of plastic litter produced in Portugal ends into open landfills (PlasticsEurope, 2020). With a program that started in 2016, the European Union Land fill Directive (1993/31/EC) is aiming to progressively reduce the volume of municipal waste entering landfills, through the closing of open-air landfills or their replacement by covered waste management facilities. Although it is likely that the European Union Land fill

Directive will have important benefits for aquatic birds in Portugal, no signs of this transition have yet been observed (PlasticsEurope, 2017, 2020).

As global plastic contamination continues to rise and the incidence of anthropogenic litter, particularly plastics, in seabirds continues to increase, studies such as ours are fundamental to evaluating the functionality of management and conservation actions. Such data are key for larger syntheses aimed at assessing current differences among areas and changes through time, particularly for Portuguese and southern Europe monitoring programs for which information is scarce or non-existent.

ACKNOWLEDGMENTS

This paper forms part of the MSc thesis of S. Rao (2020).

CONFLICTS OF INTERESTS

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support this study will be shared upon reasonable request to the corresponding author.

DECLARATION OF FUNDINGS

This research was funded by Foundation for Science and Technology (FCT - MEC, Portugal (grant number: UIDB/04326/2020) and by the National Research Foundation of South Africa (grant number: 64801).

REFERENCES

Acampora, H., Lyashevska, O., Andries, J., Franeker, V., and O'connor, I. (2016). *The use of beached bird surveys for marine plastic litter monitoring in Ireland*. https://doi.org/10.1016/j.marenvres.2016.08.002

Almroth, C. B., and Eggert, H. (2019). Marine plastic pollution: Sources, impacts, and policy issues. *Review of Environmental Economics and Policy*, *13*(2), 317–326. https://doi.org/10.1093/reep/rez012

Amaral, S. D. da S. (2009). *A avifauna como meio de valorização turística da Ria Formosa - Faro*. http://sapientia.ualg.pt/handle/10400.1/628

Anderson, M. J. (2006). Distance-based tests for homogeneity of multivariate dispersions. *Biometrics*, *62*(1), 245–253. https://doi.org/10.1111/j.1541-0420.2005.00440.x

Araújo, A., Elias, G., Reino, L., and Silva, T. (1998). Cegonha branca Ciconia ciconia. *Atlas Das Aves Invernantes Do Baixo Alentejo. Sociedade Portuguesa Para o Estudo Das Aves.*, 82–83.

Avery-Gomm, S., O'Hara, P. D., Kleine, L., Bowes, V., Wilson, L. K., and Barry, K. L. (2012). Northern fulmars as biological monitors of trends of plastic pollution in the eastern North Pacific. *Marine Pollution Bulletin*, *64*(9), 1776–1781. https://doi.org/10.1016/j.marpolbul.2012.04.017

Avery-Gomm, S., Provencher, J. F., Morgan, K. H., & Bertram, D. F. (2013). Plastic ingestion in marine-associated bird species from the eastern North Pacific. *Marine Pollution Bulletin*, 72(1), 257-259. https://doi.org/10.1016/j.marpolbul.2013.04.021

Aves — ICNF. (2018). http://www2.icnf.pt/portal/pn/biodiversidade/patrinatur/lvv/lista-aves [Accessed: May 8, 2020]

Baak, J. E., Provencher, J. F., and Mallory, M. L. (2020). Plastic ingestion by four seabird species in the Canadian Arctic: Comparisons across species and time. *Marine Pollution Bulletin*, *158*, 111386. https://doi.org/10.1016/j.marpolbul.2020.111386

Basto, M. N., Nicastro, K. R., Tavares, A. I., Mcquaid, C. D., Casero, M., Azevedo, F., and Zardi, G. I. (2019). Plastic ingestion in aquatic birds in Portugal. *Marine Pollution Bulletin*, *138*(November 2018), 19–24. https://doi.org/10.1016/j.marpolbul.2018.11.024

Battisti, C., Staffieri, E., Poeta, G., Sorace, A., Luiselli, L., and Amori, G. (2019). Interactions between anthropogenic litter and birds: A global review with a 'black-list' of species. *Marine Pollution Bulletin*, *138* (September 2018), 93–114.

https://doi.org/10.1016/j.marpolbul.2018.11.017

Belant, J. L., Ickes, S. K., and Seamans, T. W. (1998). Importance of landfills to urban-nesting herring and ring-billed gulls. *Landscape and Urban Planning*, *43*(1–3), 11–19. https://doi.org/10.1016/S0169-2046(98)00100-5

Bergmann, M., Gutow, L., and Klages, M. (2015). Marine anthropogenic litter. Springer Nature. https://doi.org/10.1007/978-3-319-16510-3

Blanco, G. (1996). Population dynamics and communal roosting of White Storks foraging at a spanish refuse dump. *Waterbirds*, *19*(2), 273–276. https://doi.org/10.2307/1521871

Bond, A. L. (2016). Diet Changes in Breeding Herring Gulls (*Larus argentatus*) in Witless Bay, Newfoundland and Labrador, Canada, over 40 Years. *Waterbirds*, *39*(sp1), 152–158. https://doi.org/10.1675/063.039.sp115

Bond, A. L., Provencher, J. F., Daoust, P. Y., and Lucas, Z. N. (2014). Plastic ingestion by fulmars and shearwaters at Sable Island, Nova Scotia, Canada. *Marine Pollution Bulletin*. https://doi.org/10.1016/j.marpolbul.2014.08.010

Bond, A. L., Provencher, J. F., Elliot, R. D., Ryan, P. C., Rowe, S., Jones, I. L., Robertson, G. J., and Wilhelm, S. I. (2013). Ingestion of plastic marine debris by Common and Thick-billed Murres in the northwestern Atlantic from 1985 to 2012. *Marine Pollution Bulletin*, *77*(1–2), 192–195. https://doi.org/10.1016/j.marpolbul.2013.10.005

Cadée, G. C. (2002). Seabirds and floating plastic debris. *Marine Pollution Bulletin*, *44*(11), 1294–1295. https://doi.org/10.1016/S0025-326X(02)00264-3

Calado, J., Veríssimo, S., Paiva, V., Ramos, R., Vaz, P., Matos, D., Pereira, J., Lopes, C., Oliveira, N., Quaresma, A., Ceia, F., Velando, A., Ramos, J. (2021) Influence of fisheries on the spatio-temporal feeding ecology of gulls along the western Iberian coast. Marine Ecology Progress Series 661:187– 201. doi: 10.3354/meps13601

Cheriak, L., Barbraud, C., Doumandji, S., & Bouguessa, S. (2014). Diet variability in the White Stork Ciconia ciconia in eastern Algeria. *Ostrich*, *85*(2), 201–204. https://doi.org/10.2989/00306525.2014.971451

Clarke, K. R. (1993). Non-parametric multivariate analyses of changes in community structure.

Australian Journal of Ecology, *18*(1), 117–143. https://doi.org/10.1111/j.1442-9993.1993.tb00438.x

Clarke, K. R., Somerfield, P. J., and Chapman, M. G. (2006). On resemblance measures for ecological studies, including taxonomic dissimilarities and a zero-adjusted Bray-Curtis coefficient for denuded assemblages. *Journal of Experimental Marine Biology and Ecology*, *330*(1), 55–80. https://doi.org/10.1016/j.jembe.2005.12.017

Codina-García, M., Militão, T., Moreno, J., and González-Solís, J. (2013). Plastic debris in Mediterranean seabirds. *Marine Pollution Bulletin*, *77*(1–2), 220–226. https://doi.org/10.1016/j.marpolbul.2013.10.002

Coulson, J. C., & Coulson, B. A. (2010). *Bird Study Lesser Black-backed Gulls Larus fuscus nesting in an inland urban colony: the importance of earthworms (Lumbricidae) in their diet*. https://doi.org/10.1080/00063650809461535

Cramp, S., and Simmons, K. (1977). *The Birds of the Western Palearctic, Vol. I*.

Djerdali, S., Guerrero-Casado, J., and Tortosa, F. S. (2016). Food from dumps increases the reproductive value of last laid eggs in the White Stork *Ciconia ciconia*. *Bird Study*, *63*(1), 107–114. https://doi.org/10.1080/00063657.2015.1135305

Duhem, C., Vidal, E., Legrand, J., and Tatoni, T. (2003). Opportunistic feeding responses of the yellow-legged gull larus michahellis to accessibility of refuse dumps. *Bird Study*, *50*(1), 61–67. https://doi.org/10.1080/00063650309461291

Farinha, J., and Costa, H. (1999). *Aves Aquáticas de Portugal-Guia de Campo*.

Gall, S. C., and Thompson, R. C. (2015). The impact of debris on marine life. *Marine Pollution Bulletin*, *92*(1–2), 170–179. https://doi.org/10.1016/j.marpolbul.2014.12.041

Gilbert, N. I., Correia, R. A., Silva, J. P., Pacheco, C., Catry, I., Atkinson, P. W., Gill, J. A., and Franco, A. M. A. (2016). Are white storks addicted to junk food? Impacts of landfill use on the movement and behaviour of resident white storks (Ciconia ciconia) from a partially migratory population. *Movement Ecology*, *4*(1), 7. https://doi.org/10.1186/s40462-016-0070-0

Gyimesi, A., Boudewijn, T. J., Buijs, R.-J., Shamoun-Baranes, J. Z., De Jong, J. W., Fijn, R.

C., Van Horssen, P. W., & Poot, M. J. M. (2016). *Bird Study Lesser Black-backed Gulls Larus fuscus thriving on a non-marine diet*. https://doi.org/10.1080/00063657.2016.1180341

Guo, H., Zheng, X., Luo, X., and Mai, B. (2020). *Leaching of brominated flame retardants (BFRs) from BFRs-incorporated plastics in digestive fluids and the influence of bird diets*. *393*(January). https://doi.org/10.1016/j.jhazmat.2020.122397

Hagemeijer, W.J.M., and Blair, M. J.(1997). *The EBCC Atlas of European Breeding Birds: Their Distribution and Abundance*. T. and A. D. Poyser, London.

Haward, M. (2018). Plastic pollution of the world's seas and oceans as a contemporary challenge in ocean governance. *Nature Communications*, *9*(1), 9–11. https://doi.org/10.1038/s41467-018-03104-3

Henry, P.Y., Wey, G. and Balança, G., 2011. Rubber band ingestion by a rubbish dump dweller, the White Stork (*Ciconia ciconia*). *Waterbirds*, 34(4), pp.504-508.

IUCN. (2018). *The IUCN red list of threatened species*; IUCN Global Species Programme Red List Unit.

Jâms, I. B., Windsor, F. M., Poudevigne-Durance, T., Ormerod, S. J., and Durance, I. (2020). Estimating the size distribution of plastics ingested by animals. *Nature Communications*, *11*(1), 1–7. https://doi.org/10.1038/s41467-020-15406-6

Kenyon, K. W., and Kridler, E. (1969). Laysan Albatrosses Swallow Indigestible Matter. *The Auk*, *86*(2), 339–343. https://doi.org/10.2307/4083505

Kwieciński, Z., Kwiecińska, H., Botko, P., Wysocki, A., Jerzak, L. and Tryjanowski, P., 2006. Plastic strings as the cause of leg bone degeneration in the White Stork (*Ciconia ciconia*). In *White Stork Study in Poland: Biology, Ecology and Conservation*. Bogucki Wydawnictwo Naukowe Poznań.

Kühn, S., Bravo Rebolledo, E. L., and Van Franeker, J. A. (2015). Deleterious effects of litter on marine life. In *Marine Anthropogenic Litter* (pp. 75–116). Springer International Publishing. https://doi.org/10.1007/978-3-319-16510-3_4

Lau, W. W. Y., Shiran, Y., Bailey, R. M., Cook, E., Stuchtey, M. R., Koskella, J., Velis, C. A., Godfrey, L., Boucher, J., Murphy, M. B., Thompson, R. C., Jankowska, E., Castillo Castillo, A., Pilditch, T. D., Dixon, B., Koerselman, L., Kosior, E., Favoino, E., Gutberlet, J. and Palardy, J. E. (2020). Evaluating scenarios toward zero plastic pollution. *Science*, *21*(1), 1–9. https://www.golder.com/insights/block-caving-a-viable-alternative/

Lindborg, V. A., Ledbetter, J. F., Walat, J. M., and Moffett, C. (2012). *Plastic consumption and diet of Glaucous-winged Gulls (Larus glaucescens)*. https://doi.org/10.1016/j.marpolbul.2012.08.020

Lopes, C. S., de Faria, J. P., Paiva, V. H., and Ramos, J. A. (2021). Characterization of anthropogenic materials on yellow-legged gull (*Larus michahellis*) nests breeding in natural and urban sites along the coast of Portugal. *Environmental Science and Pollution Research*, 36954–36969. https://doi.org/10.1007/s11356-020-09651-x

Mallory, M. L., Roberston, G. J., and Moenting, A. (2006). Marine plastic debris in northern fulmars from Davis Strait, Nunavut, Canada. *Marine Pollution Bulletin*, *52*(7), 813–815. https://doi.org/10.1016/j.marpolbul.2006.04.005

Moser, M. L., and Lee, D. S. (1992). A Fourteen-Year Survey of Plastic Ingestion by Western North Atlantic Seabirds. *Colonial Waterbirds*, *15*(1), 83. https://doi.org/10.2307/1521357

Nicastro, K. R., Lo Savio, R., McQuaid, C. D., Pedro, M., Valbusa, U., Azevedo, F., Caserod, M., Lourenço, C., and Zardi, G. I. (2018). Plastic ingestion in aquatic-associated bird species in southern Portugal. *Elsevier*. http://www.bionept.com/uploads/5/4/2/2/54224717/nicastro_et_al_2018.pdf

O’Hanlon, N. J., James, N. A., Masden, E. A., and Bond, A. L. (2017). Seabirds and marine plastic debris in the northeastern Atlantic: A synthesis and recommendations for monitoring and research. In *Environmental Pollution* (Vol. 231). https://doi.org/10.1016/j.envpol.2017.08.101

OSPAR Commission. (2008). Background document for the EcoQO on plastic particles in stomachs of seabirds. *Biodiversity Series. Publication 355*.

Peris, S. J. (2003). Feeding in urban refuse dumps: Ingestion of plastic objects by the White

Stork (Ciconia ciconia). *Ardeola*, *50*(1), 81–84.

Pinilla, J. (2000). *Manual para el anillamiento científico de aves*.

PlasticsEurope. (2017). *Plastics-the Facts 2017 An analysis of European plastics production, demand and waste data*.

PlasticsEurope. (2020). Plastics – the Facts 2020. *PlasticEurope*, 16.

Poon, F. E., Provencher, J. F., Mallory, M. L., Braune, B. M., and Smith, P. A. (2017). Levels of ingested debris vary across species in Canadian Arctic seabirds. *Marine Pollution Bulletin*, *116*(1–2). https://doi.org/10.1016/j.marpolbul.2016.11.051

Provencher, J., Bond, A., Avery-gomm, S., Borrelle, S., Bravo Rebolledo, E., Hammer, S., Kühn, S., Lavers, J., Mallory, M., Trevail, A., and Van Franeker, J. (2017). Quantifying ingested debris in marine megafauna: A review and recommendations for standardization. In *Analytical Methods* (Vol. 9, Issue 9). https://doi.org/10.1039/c6ay02419j

Provencher, J. F., Gaston, A. J., and Mallory, M. L. (2009). Evidence for increased ingestion of plastics by northern fulmars (Fulmarus glacialis) in the Canadian Arctic. *Marine Pollution Bulletin*, *58*(7), 1092–1095. https://doi.org/10.1016/j.marpolbul.2009.04.002

Puskic, P. S., Lavers, J. L., and Bond, A. L. (2020). A critical review of harm associated with plastic ingestion on vertebrates. *Science of the Total Environment*, *743*. https://doi.org/10.1016/j.scitotenv.2020.140666

Ramírez, I., Geraldes, P., Meirinho, A., Amorim, P., and Paiva, V. (2008). *Áreas marinhas importantes para as aves em Portuga [Important Areas for Seabirds in Portugal]. Projecto LIFE=04NAT/PT/000213-Sociedade Portuguesa Para o Estudo das Aves, Lisbon.*

Richardson, B. J., Avery-gomm, S., Provencher, J. F., Morgan, K. H., and Bertram, D. F. (2013). Plastic ingestion in marine-associated bird species from the eastern North Pacific. *Marine Pollution Bulletin*, *72*(1), 257–259. https://doi.org/10.1016/j.marpolbul.2013.04.021

Rodríguez, A., Ramírez, F., Carrasco, M.N. and Chiaradia, A., 2018. Seabird plastic ingestion differs among collection methods: Examples from the short-tailed shearwater.

Environmental Pollution, 243, pp.1750-1757.

Roman, L., Hardesty, B. D., Hindell, M. A., and Wilcox, C. (2019). A quantitative analysis linking seabird mortality and marine debris ingestion. *Scientific Reports*, *9*(1). https://doi.org/10.1038/s41598-018-36585-9

Roman, L., Lowenstine, L., Maeve, L., Wilcox, C., Denise, B., Gilardi, K., and Hindell, M. (2019). *Science of the Total Environment Is plastic ingestion in birds as toxic as we think ? Insights from a plastic feeding experiment*. *665*, 660–667. https://doi.org/10.1016/j.scitotenv.2019.02.184

Roman, L., Schuyler, Q. A., Hardesty, B. D., and Townsend, K. A. (2016). Anthropogenic Debris Ingestion by Avifauna in Eastern Australia. *PLoS ONE*, *11*(8), 1–10. https://doi.org/10.5061/dryad.p48f7

Rosa, G., Encarnacao, V., Leao, F., and Pacheco, C. (2009). Recenseamentos da populacao invernante de Cegonha-Branca Ciconia ciconia em Portugal (1995–2008). *VI Congresso de Ornitologia Da SPEA, IV Congresso Ibérico de Ornitologia.*

Ryan, P. G. (1988). Effects of ingested plastic on seabird feeding: Evidence from chickens. *Marine Pollution Bulletin*, *19*(3), 125–128. https://doi.org/10.1016/0025-326X(88)90708-4

Ryan, P. G. (2018). Entanglement of birds in plastics and other synthetic materials. *Marine Pollution Bulletin*, *135*(June), 159–164. https://doi.org/10.1016/j.marpolbul.2018.06.057

Sazima, I., and D’angelo, G. (2015). Handling and intake of plastic debris by Wood Storks at an urban site in South-eastern Brazil: possible causes and consequences. In *Article in North-Western Journal of Zoology*. http://biozoojournals.ro/nwjz/index.html

Schwemmer, P., & Garthe, S. (2005). At-sea distribution and behaviour of a surface-feeding seabird, the lesser black-backed gull Larus fuscus, and its association with different prey The Coastal Observing System for Northern and Arctic Seas (COSYNA) View project. *MARINE ECOLOGY PROGRESS SERIES Mar Ecol Prog Ser*, *285*, 245–258. https://doi.org/10.3354/meps285245

Seif, S., Provencher, J. F., Avery-Gomm, S., Daoust, P. Y., Mallory, M. L., and Smith, P. A.

(2018). Plastic and Non-plastic Debris Ingestion in Three Gull Species Feeding in an Urban Landfill Environment. *Archives of Environmental Contamination and Toxicology*, *74*(3), 349–360. https://doi.org/10.1007/s00244-017-0492-8

Tanaka, K., Watanuki, Y., Takada, H., Ishizuka, M., Yamashita, R., Kazama, M., Hiki N., Kashiwada, F., Mizukawa K., Mizukawa, H., Hyrenbach, D., Hester, M., Ikenaka, Y., Nakayama, S.M.M. (2020) In vivo accumulation of plastic- derived dhemicals into seabird tissues. *Current Biology* 30:723- 728.e3. doi: 10.1016/j.cub.2019.12.037

Tortosa, F. S., Caballero, J. M., and Reyes-López, J. (2002). Effect of rubbish dumps on breeding success in the White Stork in Southern Spain. *Waterbirds*, *25*(1), 39–43. https://doi.org/10.1675/1524-4695(2002)025[0039:eordob]2.0.co;2

Tsachalidis, E. P., & Goutner, V. (2002). Diet of the White Stork in Greece in relation to habitat. *Waterbirds*, *25*(4), 417–423. https://doi.org/10.1675/1524-4695(2002)025[0417:dotwsi]2.0.co;2

UNEP. (2016). The Emissions Gap Report 2014: A UNEP synthesis Report. http://www.unep.org/pdf/SEI.pdf

Van Franeker, J. A. (2004). *Save the North Sea Fulmar-Litter-EcoQO Manual Part 1: Collection and dissection procedures*. www.alterra.wur.nl

Van Franeker, J. A., Blaize, C., Danielsen, J., Fairclough, K., Gollan, J., Guse, N., Hansen, P. L., Heubeck, M., Jensen, J. K., Le Guillou, G., Olsen, B., Olsen, K. O., Pedersen, J., Stienen, E. W. M., and Turner, D. M. (2011). Monitoring plastic ingestion by the northern fulmar Fulmarus glacialis in the North Sea. *Environmental Pollution*, *159*(10), 2609–2615. https://doi.org/10.1016/j.envpol.2011.06.008

Van Franeker, J. A., and Law, K. L. (2015). Seabirds, gyres and global trends in plastic pollution. *Environmental Pollution*, *203*, 89–96. https://doi.org/10.1016/j.envpol.2015.02.034

Velez, N., Zardi, G.I., Savio, R.L., McQuaid, C.D., Valbusa, U., Sabour, B. and Nicastro, K.R., 2019. A baseline assessment of beach macrolitter and microplastics along northeastern Atlantic shores. *Marine Pollution Bulletin*, 149, p.110649.

Velez, N., Nicastro, K.R., McQuaid, C.D. and Zardi, G.I., 2020. Small scale habitat effects on anthropogenic litter material and sources in a coastal lagoon system. *Marine Pollution Bulletin*, 160, p.111689.

Vrezec, A. (2009). Insects in the White Stork Ciconia ciconia diet as indicators of its feeding conditions: the first diet study in Slovenia. *Acrocephalus*, *30*(140), 25–29. https://doi.org/10.2478/v10100-009-0003-8

Weiser, E. L., and Powell, A. N. (2011). Reduction of Garbage in the Diet of Nonbreeding Glaucous Gulls Corresponding to a Change in Waste Management. In *ARCTIC* (Vol. 64, Issue 2).

Wilcox, C., Van Sebille, E., Hardesty, B. D., and Estes, J. A. (2015). Threat of plastic pollution to seabirds is global, pervasive, and increasing. *Proceedings of the National Academy of Sciences of the United States of America*, *112*(38), 11899–11904. https://doi.org/10.1073/pnas.1502108112

FIGURE LEGENDS

Figure 1. Proportion (%) of (A) White Stork *Ciconia ciconia* (n = 52); (B) Northern Gannet *Morus bassanus* (n = 56); (C) Lesser Black-backed Gull *Larus fuscus* (n =143); (D) Yellow-legged Gull *Larus michahellis* (n = 181) which ingested anthropogenic litter (total plastic and non-plastic items).

Figure 2. Mean (+SD) individual plastic ingested by White Stork *Ciconia ciconia* in (A) counts and (B) mass; Northern Gannet *Morus bassanus* (C) counts and (D) mass; Lesser Black-backed Gulls *Larus fuscus* in (E) counts and (F) mass; Yellow-legged Gull *Larus michahellis* in (G) counts and (H) mass.

Figure 3. Two-dimensional non-metric multidimensional scaling (MDS) for ingested plastic counts with data grouped by (A) species or (B) year interval. PERMANOVA main results: The

pattern of ingestion by *C. ciconia* significantly differed from that of the other species while the period 2014-2015 was significantly different from the other time frames.

Figure 4. Two-dimensional non-metric multidimensional scaling (MDS) for ingested plastic mass when data grouped by (A) species or (B) year interval. PERMANOVA main results: the pattern of ingestion by *C. ciconia* significantly differed from that of the other species.

Table 1. Frequency of occurrence of plastics (95% confidence intervals – CI), number and mass of plastics ingested by White Stork *Ciconia ciconia* (n = 110).

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

Table 2. Frequency of occurrence of plastics (95% confidence intervals – CI), number and mass of plastic ingested by Northern Gannet *Morus bassanus* (n = 137).

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

Table 3. Frequency of occurrence of plastics (95% confidence intervals – CI), number and mass of plastic ingested by Lesser Black-backed Gull *Larus fuscus* (n=175).

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

Table 4. based on plastic categories. Frequency of occurrence of plastics (95% confidence intervals – CI), counts and mass by Yellow-legged Gull *Larus michahellis* (n=70).

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