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COIMBRA

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WATERBIRDS AS SENTINELS OF MICROPLASTIC AND PLASTIC ADDITIVE POLLUTION IN COASTAL SYSTEMS IN SOUTHERN PORTUGAL

Dissertação no âmbito do Mestrado em Ecologia orientada pela Doutora Ana Cláudia do Souto Gonçalves Norte (Departamento de Ciências da Vida da Universidade de Coimbra e MARE – Marine and Environmental Sciences Centre) e Professor Doutor Jaime Albino Ramos (Departamento de Ciências da Vida da Universidade de Coimbra e MARE – Marine and Environmental Sciences Centre) e apresentada ao Departamento de Ciências da Vida da Universidade de Coimbra

Julho 2022

Em memória de Rui Manuel de Matos
28/06/1957 - 22/04/2021

WATERBIRDS AS SENTINELS OF MICROPLASTIC AND PLASTIC ADDITIVE POLLUTION IN COASTAL SYSTEMS IN SOUTHERN PORTUGAL

AVES AQUÁTICAS COMO SENTINELAS DE POLUIÇÃO POR MICROPLÁSTICOS E ADITIVOS DE PLÁSTICOS EM SISTEMAS COSTEIROS NO SUL DE PORTUGAL

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Universidade de Coimbra
Coimbra | 2022

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FACULDADE DE
CIÊNCIAS E TECNOLOGIA
UNIVERSIDADE Ð
COIMBRA

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Thesis Title: Waterbirds as sentinels of microplastic and plastic additive pollution in coastal systems in southern Portugal

Keywords:

- Plastic pollution;
- Plastic ingestion;
- PBDE;
- Shorebirds;
- Coastal birds.

Acknowledgements / Agradecimentos

Não poderia passar esta oportunidade para agradecer a todas as pessoas que de uma forma ou doutra me ajudaram ao longo destes últimos 2 anos. Como tudo na vida, estes tiveram os seus altos e baixos, no entanto, tenta-se sempre tirar o máximo proveito dos melhores momentos.

Queria começar por dar um forte agradecimento à Dra. Ana Claúdia Norte e ao Prof. Dr. Jaime Ramos. Esta tese nunca teria alcançado este patamar sem o vosso olhar atento, expertise e dedicação. Todos os comentários e concelhos contribuíram tanto para a tese, como para comigo e para a minha formação como futuro profissional. Obrigado pelas oportunidades proporcionadas ao longo destes 2 anos e pela oportunidade de integrar um grupo como o ECOTOP MARE-UC. São ambos, para mim, uma inspiração!

Um agradecimento é também devido ao Dr. Vítor Paiva, pela simpatia, por toda a ajuda a nível de analises estatísticas e pelo *guidance* no laboratório. Às vezes encontrar o material de laboratório necessário nem sempre é tão fácil quanto parece!

À Sara Veríssimo, pela ajuda em laboratório, pelas análises, recolha das amostras, por estar sempre disponível para me tirar dúvidas, e no geral, por toda a sua dedicação para que não faltasse nada para esta tese.

À Diana Matos por todo o acompanhamento ao longo do desenvolvimento dos trabalhos de laboratório e constante olhar atento.

Ao Vítor Silva, pela amizade, companheirismo e momentos passados, pela ajuda durante o processamento das amostras, e por todas as idas à Figueira de carro. Ficam as memórias do gelado do Mac no areal da Figueira da Foz, e a procura de uma corda numa falésia ao pé da CIMPOR.

A toda a equipa do ECOTOP pela simpatia e bom ambiente que proporcionam todos os dias. O meu mais sincero obrigado!

Aos meus colegas de mestrado, que fizeram com que o 1º ano passasse a voar. Obrigado!

Não poderia deixar de agradecer também todas as pessoas que diariamente estiveram sempre presentes e disponíveis para me aturar a qualquer hora.

À minha família, em especial aos meus pais. À minha mãe por todo o apoio, carinho e confiança que deposita em mim e ao meu pai por sempre, desde criança, fomentar a minha curiosidade e o querer saber sempre mais. O vosso apoio diário ajudou-me a ultrapassar muito, até mesmo coisas dos quais por vezes não falo. Ambos tornaram isto tudo possível. Sei que sem os vossos sacrifícios nunca teria chegado a onde estou.

Ao resto da minha família mais chegada, em especial ao Lucas, o meu irmão de outra mãe que esteve sempre presente em todos os momentos. Estiveram todos sempre lá para mim. Obrigado por serem a família que são e por sempre me terem apoiado incondicionalmente.

À minha Inês, por sempre estar presente quando eu mais precisava, nos momentos em que batia no fundo, e nos momentos em que atingia picos. Todo o amor, companheirismo, carinho e paciência que tiveste para me aturar ao longo dos últimos 2 anos são irretribuíveis.

Aos meus amigos, Alberto, Valente, Miguel, Elmer. Estiveram sempre presentes, nem que fosse para uma jogadita ao fim de um dia de trabalho. Obrigado pelas horas infindáveis de bons momentos!

Aos meus amigos de longa data, ao Rodolfo e ao Ricardo, embora longe estavam sempre perto, nem que fosse com uma mensagem de vez em quando a saber como corriam as coisas.

A todos os outros, que para esta tese também contribuíram. O meu sincero obrigado!

Por fim, dedico esta tese ao meu tio, Rui Manuel de Matos Gomes. Não há um único dia em que não pense em ti. Obrigado por em vida teres sido a pessoa que eras.

“*As long as we remember a person, they’re not really gone.*”
– Justin Cronin

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Abstract

Plastics are an indispensable part of our everyday lives. From plastic bags to keyboards, plastics can be found in almost all everyday items. When discarded plastics reach the environment, they progressively degrade into smaller pieces over time, eventually originating microplastics. These particles are smaller than 5mm and can now be found in almost every environment. Most microplastics carry their non-chemically bound additive, a chemical substance used to enhance certain characteristics of the plastic. Polybrominated diphenyl ethers (PBDE) are a class of additives used as flame retardants, currently regulated by the European Union. PBDEs at certain thresholds have been reported to have deleterious health implications in the exposed individuals. Due to their prevalence in the environment, microplastics and their additives are ingested by a multitude of organisms. Waterbirds (shore/coastal birds and seabirds) are known to be exposed to this type of pollution either directly or indirectly. The southern part of Portugal is no exception to plastic and additive pollution. However, current knowledge gaps do not allow us to answer most of the monitoring and pollution assessment challenges posed by both types of contamination. Thus, to bridge some of these knowledge gaps a first descriptive analysis of plastics found in the faeces and regurgitates, and the additive concentrations (PBDE 28,47, 99, 153, 154, 183) found in the eggs of 6 waterbird species sampled in Ria Formosa (Algarve), was performed: Yellow-legged gull (*Larus michahellis*); Black-winged stilt (*Himantopus himantopus*); Pied avocet (*Recurviostra avossetta*); Kentish plover (*Charadrius hiaticula*); Little tern (*Sternula abifrons*); Audouin's gull (*Ichthyaetus audouinii*). The overall goal was to establish a gradient of microplastic and additive contamination across these species, in order to obtain an overall view of contamination in these coastal systems and to further help decide which of the 6 species can better suit the role of a sentinel in the future. Overall, 647 (micro)plastics were found (307 in the faeces and 336 in regurgitates). The majority were microplastics, mainly fibres and fragments. A large proportion were blue, transparent, red and brown. Analysis of the polymers resorting to µ-FTIR was not a possibility due to time constraints. No correlation was found between the amount of (micro)plastics found in the faecal samples and the additives assimilated in the eggs. However, all target PBDEs were prevalent in the eggs and in similar concentrations and proportions across species, indicating that their presence is now ubiquitous in estuarine and coastal environments and widespread in different species of top predators. Furthermore, a decreasing gradient of (micro)plastic contamination along an estuarine-sea gradient could not be confirmed. Yet, the declining frequencies of occurrence and quantity of overall plastics found suggests that, with current data, we cannot exclude the existence of such gradient. Comparing species that foraged more inland and those that foraged in transition zones, or nearer to the shore, has shown that there is indeed a downwards trend. Likewise, comparing the regurgitates of a coastal species with those that tend to have pelagic habits has revealed similar results. The (unexpected) high amount of plastics found in some of the species revealed that, similar to the additives, plastic pollution is now widespread, suggesting a growing pervasiveness. Evaluating plastic contamination and considering intrinsic characteristics of the different species allowed us to take a first step towards the identification of possible species that, in the future, can be used as efficient sentinel species to assess, some of the still unknown, spatial and temporal trends of (micro)plastic and additive pollution in coastal and estuarine environments.

Keywords: Plastic pollution; Plastic ingestion; PBDE; Coastal Birds; Shorebirds.

Resumo

Os plásticos são uma parte indispensável do nosso quotidiano. Dos sacos de plástico aos teclados, os plásticos estão presentes em quase todos os objetos do nosso dia a dia. Quando presentes no ambiente, os plásticos podem, ao longo do tempo, começar a degradar-se em inúmeros pedaços levando a uma redução gradual do seu tamanho e eventualmente originando microplásticos. Estas partículas de plástico medem menos de 5mm e podem atualmente ser encontradas em quase todos os ambientes e meios. A maioria dos microplásticos carrega aditivos não quimicamente ligados. Estas substâncias químicas são utilizadas para conferir certas características aos plásticos. Os éteres difenílicos polibromados (PBDE) são uma classe de aditivos usados como retardantes de chamas, atualmente regulamentados na União Europeia, e que, quando presentes a certas concentrações têm implicações deletérias na saúde dos indivíduos expostos. Devido à sua prevalência e persistência no ambiente, ambos os contaminantes podem potencialmente ser ingeridos por diferentes organismos. Sabe-se que as aves aquáticas (aves costeiras, limícolas e oceânicas) estão expostas a este tipo de poluição de uma forma direta e/ou indiretamente. A zona sul de Portugal não é exceção à problemática da poluição por plásticos e aditivos, no entanto, as atuais lacunas de conhecimento não nos permitem responder à maioria dos desafios de monitorização impostos por este tipo de poluição. Assim, para colmatar algumas destas lacunas foi realizada pela primeira vez uma análise descritiva dos (micro)plásticos encontrados em fezes e regurgitos, de 6 espécies de aves aquáticas amostradas na Ria Formosa (Algarve) (Gaivota-de-patas-amarelas (*Larus michahellis*); Pernilongo (*Himantopus himantopus*); Alfaiate (*Recurviostra avossetta*); Borrelho-de-coleira-interrompida (*Charadrius hiaticula*); Chilreta (*Sternula abifrons*); Gaivota de Audouin (*Ichthyaetus audouinii*)). Adicionalmente, foi ainda descrito as concentrações de aditivos (PBDE 28,47, 99, 153, 154, 183) encontrados nos seus ovos. O objetivo geral passou por obter uma visão geral da contaminação por (micro)plásticos e aditivos destas espécies, em ambientes costeiros e estuarinos, de modo a auxiliar no futuro o estabelecimento de uma(s) destas 6 espécies como monitor de poluição na Ria Formosa. No total, 647 plásticos foram encontrados (307 em amostras de fezes e 336 em regurgitos). A maioria eram fibras e fragmentos, e tinham o tamanho de microplásticos. Uma grande porção eram de cor azul, transparente, vermelho e castanho. Devido a limitações de tempo, não foi possível realizar análises dos polímeros usando µ-FTIR.

Nenhuma correlação foi encontrada entre o número de plásticos nas amostras de fezes e os aditivos assimilados nos ovos. Porém, todos os PBDE que foram alvo do estudo foram encontrados em concentrações e proporções similares nos ovos das espécies alvo, indicando a sua presença e persistência em ambientes estuarinos e costeiros, tal como, em diferentes predadores de topo. Um gradiente decrescente de contaminação por (micro)plásticos nas fezes das espécies ao longo de um gradiente estuário-mar não pôde ser totalmente confirmado. No entanto, o facto de as frequências de ocorrência e quantidades gerais de plásticos ao longo do gradiente serem decrescentes, faz com que este gradiente não possa ser totalmente descartado. Ao comparar as fezes de espécies que foragiam zonas mais interiores dos estuários com espécies que exploram zonas mais costeiras, é notável uma tendência decrescente. Similarmente, ao comparar-se os regurgitos de uma espécie costeira com uma com hábitos tendencialmente pelágicos, demonstrou-se os mesmos resultados. As (inesperadas) elevadas quantidades de plásticos encontradas em algumas das espécies revelam, que tal como os aditivos, estes encontram-se já disseminados em vários ambientes. A avaliação do consumo de plástico de cada espécie e as suas características intrínsecas, permitiu-nos dar um primeiro passo para identificar possíveis espécies que podem, no futuro, ser consideradas espécies sentinelas para a avaliar algumas das ainda desconhecidas tendências espácio-temporais da poluição por (micro)plásticos e os seus aditivos em ambientes costeiros e estuarinos.

Palavras-chave: Poluição por plásticos; Ingestão de plásticos; PBDE;
Aves aquáticas; Aves limícolas.

Contributions / Contribuições

I declare that this thesis was written and organized by me (the author), and I confirm that it has not been previously submitted, in whole or in part, to obtain another academic degree. I confirm that the work described was mostly done by me, and other contributions are clearly acknowledged in the text with appropriate citations/references. I performed all laboratory work except for the majority of the sample collection, which was performed by Sara Veríssimo, the analyses of the additives (PBDE) which were performed by Sara Veríssimo and Diana Matos and the initial sorting and extraction of plastics from the regurgitates of both gull species (Audouin's gull and Yellow-legged gull) which was done by Lara Cerveira. I wrote the first draft of the paper and incorporated further suggestions from both supervisors.

Declaro que esta tese foi elaborada por mim (o autor) e confirmo não ter sido previamente submetida, total ou parcialmente, para obtenção de outro grau académico. Confirmo que o trabalho descrito foi na sua maioria realizado por mim, sendo que outras contribuições estão claramente reconhecidas no texto com as devidas citações/referências. Realizei todo o trabalho laboratorial, exceto a recolha de amostras que fora realizada na sua maioria pela Sara Veríssimo, a análise dos aditivos (PBDE) que fora realizado pela Sara Veríssimo e Diana Matos e a extração de plásticos dos regurgitos de ambas as espécies de gaivota que fora realizado pela Lara Cerveira. Escrevi a primeira versão do artigo e incorporei as posteriores sugestões de ambos os orientadores.

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Table S12 – Generalized Linear models used to assess the effect of bird species (HH – Black-winged stilt, RA – Pied avocet, CA – Kentish plover, SA – Little tern, IA – Audouin's gull, LM – Yellow-legged gull) on presence and number of plastics and their shapes (fibre) and colours (blue) in regurgitates. Each model used is identified on the left. Associated ß±SE, Z and p values are presented. Highlighted in red are significant values (<0.05). At the bottom, zero inflation tests (ratio) and performance of the models performed on quantitative data are shown. Highlighted in red are the models selected in each case.

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Chapter I – Introduction

1.1 - Plastics in our everyday lives

It is almost unimaginable to live in a world where plastics do not exist. Their versatility in different areas and overall usefulness, have made them a core component of most of our everyday items. Plastics are currently used to make food packages, fishing nets, plastic tubes, hospital material, cooking ware, face masks and thousands of other items, effectively making them an indispensable part of our lives. This has led to a surge in demand, boosting production to an almost exponential rate since their first commercialization's in the 1950's (Plastics Europe, 2013). Data from 2020 estimated that 367 million tons of plastic were produced worldwide per year, where Europe contributed with 55 million tons (Plastics Europe, 2021). Current estimates show that around 8300 million tons of plastic have been produced so far. Of these, only 600 million tons were recycled (≈7%) and approximately 4600 million tons (55%) went directly to landfills (Geyer et al., 2017). Around 8 million tons are thought to reach the ocean every year (Gallo et al., 2018) with rivers being responsible for the transport and input of 1.15 to 2.41 million tons/year into coastal systems (Lebreton., et al 2017). Current models and trends, show that in coastal and marine systems these numbers have reached a plateau, stabilizing in recent years until 2019. However, plastic pollution appears to be reaching more remote areas with low or even non-existent anthropogenic presence, thus spreading and prevailing in almost every place in the world (Galgani, 2021).

The prevalence of plastics in the environment combined with scientific papers and news articles revealing contamination by (micro)plastics in humans (Barboza et al., 2018; Ragusa et al., 2021; Leslie et al., 2022) and anti-plastic and plastic pollution awareness movements have led to a higher awareness and subsequent scrutinization of plastics by the mainstream public. For the past 20 years this higher awareness, has shed light into this worldwide problem that was first described in scientific papers in the early days of ecology research in the 60's and 70's (Rochman., 2020).

1.2 - Plastics

Plastics are a man-made synthetic or semi-synthetic material mainly composed by a synthetic polymer made of carbon monomers and other atoms such as oxygen, sulphur, and nitrogen, that comprise up to 90% of the total mass of the plastic (Andrady & Neal., 2009). In Europe, the most common polymer types currently used and manufactured are polyethylene (PE-low density and high density), polypropylene (PP) and polyvinyl chloride (PVC) (Plastics Europe 2021). The remaining mass of the plastic is typically composed by a chemical known as an additive. Plasticizers and brominated flame retardants are the most commonly used additives, to enhance some utilitarian characteristics of the plastics (see below).

Most synthetic polymers (also referred as plastics) are not biodegradable (Geyer et al., 2017), thus persisting in the environment for extended periods of time (Barnes et al 2009). Due to different environmental stressors, plastics are subjected to various physical mechanisms of degradation such as wave and current motion, photodegradation and other mechanisms of abrasion which eventually lead to a progressive fragmentation into smaller pieces (Arthur et al 2009; Ribeiro et al., 2019). As a result, a plastic classification method according to the size of the fragment was developed to better classify and standardize plastic description in scientific studies. The classification lists plastic particles as follows: macroplastics (> 20 mm diameter), mesoplastics (5-20 mm diameter), microplastics (MPs, < 5 mm diameter) and nanoplastics (NPs, from 1 μm to 1 nm) (GESAMP, 2016). According to this classification, the commonest class of plastics, regarding the total number of particles in the environment, are microplastics, while macro and mesoplastics dominate in terms of overall weight (Gunaalan et al., 2020). Latest conservative estimates reveal that, in the ocean alone, there are around 24.4 trillion microplastic pieces weighting between 82 000 to 578 000 tons (Isobe et al., 2021). Additionally, microplastics can also be classified according to their origin and relative shape, as shown in Fig. 1.

Waterborne microplastic pollution around the globe is mainly influenced by anthropogenic factors such as the presence of large cities or industrial areas near rivers or oceans, and maritime activities such as fishing and tourism attractions (Cole et al. 2011). Due to this, plastics tend to accumulate in the northern hemisphere (mainly near the tropics) where most developed countries are located. Coincidently, these are the areas where the amount of people living near coastal ecosystems is considerably higher (Barnes et al., 2009; Erisken et al., 2014; Galgani et al., 2015). Yet, human presence is not the only factor impacting microplastic distribution. Some abiotic factors can also play a role. The hydrodynamics of the area are deeply intertwined with its distribution. Salinity, surface-water, and deep-water currents convergence (gyres) in saltwater systems dictate, most of the times, areas of plastic accumulation (hotspots) (Barnes et al., 2009; Erisken et al., 2014; Lourenço et al., 2017). In brackish and freshwater systems, further abiotic factors such as rainfall rates and depth can also contribute to its distribution (Cohen et al., 2019; Krelling and Turra., 2019). However, in this case, there are still complex spatio-temporal patterns that are still not fully understood (Krelling and Turra., 2019).

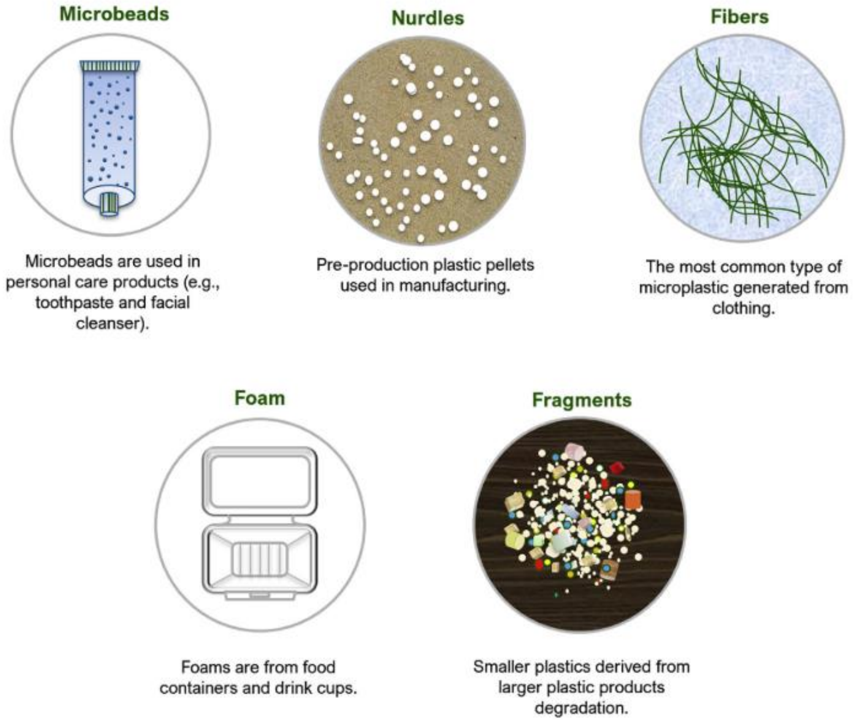

Figure 1 – Microplastic classification according to their origin and relative shape. In Wu et al. (2019).

While waterborne, (micro)plastics will either: stay at the surface and reach longer distances; or progressively sink and deposit to the sediment (Moore, 2008; Hidalgo-Ruz et al., 2012; Frias et al., 2016). Progressive sinkage of these particles can occur naturally due to the density of their synthetic polymer (PP and PE often sink due to their higher density, Browne et al., 2010), or with the help of biofilm producing bacteria which can colonize the particles and form biofilms that increase their density, thereby making the particles sink (Ioakeimidis et al., 2019). In seawater, microplastics, progressively sink towards the seabed or sediment where they are found in larger quantities (range 0.21 to more than 77 000 items/m^2) when compared to that of the surface or the water column (range 8×10^{-5} to 5 items/m^2 - Hidalgo-Ruz et al., 2012). By contrast, in rivers, microplastics will mostly travel at the surface or in the water column, (Browne et al 2010) depending, mostly, on the current speed. Accordingly, areas with slower current velocities have higher amounts of microfibers in the sediment (Lourenço et al., 2017).

Thus, estuaries that have periodic low hydrodynamics during low-high tide switch, are likely to function as hotspots for all types of plastics, as the stillness of the water during tide switch allows for the particles to deposit to the sediment (Woodall et al., 2014; Ouyang et al 2022). Both the seabed and intertidal/estuarine sediments are thus hypothesized to have larger amounts of microplastics, functioning as possible traps for these particles (Browne et al., 2010; Hidalgo-Ruz., et al 2012; Lourenço et al., 2017).

As microplastics eventually sink, they become more bioavailable for bottom dwelling organisms, usually, suspension feeders, deposit feeders, filter feeders and species that forage along the sediment (Messinetti et al., 2018). Most suspension and filter feeders (i.e mussels, limpets, zooplankton etc) have a still unknown, but likely limited capability, of discriminating food from non-food items, meaning that almost every particle with 1mm in diameter (or less) is likely to be ingested directly from the environment (Moore, 2008). By contrast, deposit feeders like polychaetas, have more elaborate ways of discriminating food from non-food items, indicating that in their case plastic ingestion results from a tactile or visual misidentification of their food sources (Thompson et al., 2004). Both cases can be classified as direct (or primary) consumption, where plastic particles and other chemicals are consumed directly from the environment (Moore et al., 2008). It is important to note that direct ingestion is not exclusive to lower trophic levels. Top predators can also accidently ingest microplastics directly from the environment as they either unintentionally bycatch particles or chemicals when foraging, or misidentify plastic pieces as food items (Moore, 2008).

Besides direct ingestion, secondary consumption (indirect ingestion) of (micro)plastics and their chemicals also occurs. In this case, already contaminated prey is consumed by predators higher on the food web. Trophic transfer has already been proven to occur under laboratory (Farrell and Nelson, 2013; Watts et al 2014) and semi-natural conditions at the highest trophic levels (Au et al., 2017; Nelms et al., 2018), indicating that bioaccumulation in lower trophic levels is also likely to happen (Nelms et al., 2018). For example, under laboratory conditions, shore crabs (*Carcinus maenas*) have been reported to have both assimilated microplastics directly through the gills, and through the ingestion of mussels (*Mytilus edulis*) that were previously exposed to fluorescent labelled microplastics (Watts et al 2014). The shore crabs retained the ingested microplastics inside their bodies for up to 14 days and the inhaled microplastics for up to 21 days. Furthermore, Farrell and Nelson (2013) showed that in a controlled environment, microplastics assimilated by shore crabs were present in the stomach, hepatopancreas, haemolymph, ovary and gills of the individuals, implying that microplastic translocation from ingestion to other vital organs is a possibility. In higher trophic levels in semi-natural conditions, captive grey seal scats were examined for their microplastic contents as well as the guts of wild-caught mackerel that the grey seals fed on. Results showed that frequency of occurrence (FO) in samples of grey seal scats was 48% and 32% in fish guts, suggesting a possible trophic transfer from one species to the other (Nelms et al. 2018).

Generally, trophic transfer usually implies the bioaccumulation of certain contaminants in an individual's body. Microplastics, however, do not only accumulate inside the individual (the digestive tract), but also on parts of the organism exposed to the surrounding environment. In mussels and fish, microplastics also tend to accumulate in their skin, gills and other vital organs exposed to the surrounding water (see Franzelliti et al., 2019 for a comprehensive compilation of microplastic accumulation studies in tissues). This is especially relevant since these are taxa that link different trophic levels between pelagic and benthic ecosystems and that are especially susceptible to consuming and accumulating microplastics under natural conditions (Franzellitti et al., 2019).

1.3 - Additives, PBDE's and microplastics as vectors

Even though microplastic contamination receives a lot of attention, some underlying consequences linked to this type of pollution, that have major and more prominent effects on biota, are less studied. Additives, generally represent up to 10% of the total weight of the plastic particle (Andrady & Neal., 2009) and can be classified in two large groups: plasticizers, used to enhance characteristics such as resistance, plasticity, and malleability; and flame retardants, named after its intended use. Plasticizers and flame retardants are frequently not covalently bound to the plastic matrix (Engler et al., 2012) meaning that when exposed to environmental stressors (mostly UV-radiation or digestive fluids when ingested) they detach from the matrix, leaching to the surrounding environment and eventually persisting for long periods of time (Mensah et al., 2022). Additives have numerous pathways into the environment such as: industrial and municipal wastewater discharges into freshwater systems, atmospheric deposition, runoff from landfills and agricultural areas that use sewage sludge as a fertilizer (Hermabessiere et al., 2017). A myriad of additives have been reported to be present in the environment at varying quantities, yet total amounts are hard to quantify. Some of the most commonly found in environmental analysis are phthalic acid esters (PAE - a phthalate used as a plasticizer), nonylphenols and bisphenol A (BPA - both used as antioxidant and stabilizers), and lastly, polybrominated diphenyl ethers (PBDE - a type of brominated flame retardant) (Hermabessiere et al 2017). The latter can be classified further as to their commonest commercial formulations as penta-, octa- and deca-BDE (depending on the total number of atoms of bromine in the chemical structure). PBDEs have been the subject of intense regulation worldwide including in the European Union. Plastic products that have in their composition a PBDE/weight ratio higher than 0,1% have been banned. Furthermore, tetra- to hepta-BDE's were flagged for elimination by the Stockholm convention of 2016, thus restricting their usage even further. These bans and regulations are due to PBDEs being considered toxic, persistent and as having the highest potential to bioaccumulate in living tissue. PBDEs have the highest octanol-water partition coefficients out of all other additives (Hermabessiere et al., 2017), translating into a higher potential to bioaccumulate (Net et al., 2015). The main concern is that PBDEs can act as endocrine disruptors, influencing the immune system and the reproductive function of different animals across a multitude of taxa (Gunaalan et al., 2020). Wild animals are not the only ones showing detrimental effects caused by this type of pollution. Human exposure to these compounds has also shown negative effects. Aerial PDBE concentrations commonly found in current household (BDE, 47, 99, 100 and 153) has already been detected in pregnant woman (> 95%, n=343) and have been associated with a significant decrease in fertility as PBDEs act as endocrine disruptors (Harley et al., 2010). Accordingly, microplastic abundance has been reported to have a positive relation with higher concentrations of PBDEs in the environment (Rochman et al., 2014).

Similar to microplastic ingestion, additive uptake, also occurs indirectly through the ingestion of already contaminated prey, or directly, through breathing /ingestion of contaminated medium or (micro)plastics still carrying these additives. Additionally, transfer through the trophic web in marine and coastal environments has been evidenced all the way through to top predators (Tanaka et al., 2015). Huber et al., (2015) reported the presence of PBDEs and a variety of other chemicals in the eggs of common eider (*Somateria mollissima*), European shag (*Phalacrocorax aristotelis*) and herring gull (*Larus argentatus*). Seventeen different PBDEs were targeted for analysis, and all were present across the 3 species. Effects associated with additive assimilation in top predators are still relatively unknown. Yet, there are other compounds and pathogens with well documented effects that can bond to the plastic matrix. Pollutants such as heavy metals (Brennecke et al., 2016), persistent organic pollutants, hydrophobic organic chemicals, and pathogens such as bacteria (Koelmans., et al 2016) may attach to these particles. Microplastics turn, thus, intro vectors for a wide array of pollutants and pathogens.

1.4 - Waterbirds as sentinels of plastic pollution in coastal environments

Although large pieces of plastic can have direct deleterious effects on fauna (Gregory, 2009), microplastics, per se, do not seem to pose a threat to taxa belonging to the higher levels of the food chain (Güven et al., 2017). In small or microscopic animals (larval stages of some species, for example), accumulation of small or nano plastic particles can indeed cause blockages that could lead to the individual's death (Wright et al., 2013) or to cellular modifications and disruptions (Capolupo et al., 2018) since egestion is often not possible (Wright et al., 2013). However, in larger individuals, microplastic accumulation is not likely to happen, (albeit not impossible) since most particles pass through the digestive tract without causing any type of blockage that would otherwise be fatal for the organism. Although it is possible that adherence to the walls of the digestive tract may happen, it does not been seem to cause any serious effects (Ribeiro et al., 2019). Yet, the fact that microplastics can act as vectors for a plethora of pollutants and potentially dangerous pathogens, causes concern. This concern combined with the still unknown effects of plastic additives, ongoing accumulation of (micro)plastics in coastal and marine systems (Barnes et al., 2009), and the fact that prey like fish, bivalves, crustaceans, and other filter feeders (all known to bioaccumulate microplastics and additives) constitute the majority of the diet of most waterbird species, turn seabirds and aquatic birds (shore/coastal birds) into potential targets for studies regarding (micro)plastic and additive assimilation and accumulation (Avery-Gomm et al., 2013; Lourenço et al., 2017).

Since waterbirds are top predators, integral parts of coastal ecosystems (Mallory et al., 2010), ubiquitous, susceptible to (micro)plastic and additive ingestion (due to their diet and foraging strategy), relatively abundant, easy to sample, and appealing to the public, turn them into potentially good bioindicators and conservation targets for microplastic and additive pollution (Burger and Gochfeld 2004). Additionally, most waterbird species feed on fish, crustaceans, bivalves and other seafood which are shared food sources with humans and other species. Therefore, using waterbirds as a proxy may reveal if our food sources may or may not be contaminated, thus, potentially constituting as sentinel species for microplastics and their additives (Nicastro et al., 2018; Carbery et al., 2018). As an example, the northern Fulmar (*Fulmarus glacialis*) is now used as a bioindicator and sentinel to detect (micro)plastic pollution in the North Sea. The continued surveillance of this species makes it possible to establish temporal and spatial trends of (micro)plastic and other pollutants using data from a variety of scientific publications (Avery-Gomm et al., 2018). However, a multispecies monitoring approach on the incidence of plastics is essential, to assess different parameters such as composition, amounts and trends of these contaminants, in a broader array of environments. Comparing different species might single which factors are influencing microplastic uptake and determining the usefulness (or not) of a certain species for microplastic and additive monitoring (Acampora et al., 2016). Moreover, the need to determine additional proxies is exacerbated by the fact that bivalves, mostly oysters and clams (considered as two of the most suitable proxies for plastic pollution), reveal some limitations due to their selective ingestion mechanisms and ingestion abilities, since they only ingest a specific size range of plastics (small micro and nanoplastics) (Ward et al., 2019).

For most waterbird species, few ingestion studies have been performed to date. In open ocean environments, Codina-Garcia et al. (2013) necropsied the stomach contents of 171 seabirds from 9 species. The authors reported that gull species had a lower incidence of plastic content in their stomachs (*Larus michahellis* frequency of occurrence: 33% n =12; *Ichthyaetus audouinii*: 13% n=15; *Ichthyaetus melanocephalus*: 25% n=4) when compared to shearwater species (*Calonectris borealis*: 96% n= 49; *Puffinus mauretanicus*: 70% n=46; *Puffinus yelkouan*: 71% n=31). This was not expected as some gulls are known to forage near landfills and consequently consume larger amounts of anthropogenic litter. The authors explain this variability with a larger retention time of plastics in shearwaters due to the gull's more efficient ability to regurgitate. Most plastics found were fragments smaller than 5mm, therefore classifying as microplastics. Rodrigues et al., (2016) found that in 421 corpses of Cory's shearwaters (*Calonectris borealis*), collected from 2000 to 2012 in the Azores, 93% had at least one piece of plastic on their stomach thus corroborating the data from the cory's shearwater obtained by Codina-garcia et al. (2013). Additionally, plastics had a mean size of 3.2 mm. A similar study conducted by Basto et al. (2019) sampled 288 seabirds' carcasses across 16 species. Thirty-seven individuals (12.9%) of 6 species (*L. michaellis, L. fuscus, Morus bassanus, Chroiocephalus ridibundus, Phalacrocorax carbo, Rissa tridactyla*) were found with plastics in their stomachs. Of the plastics found in each individual, 66% to 100% were microplastics. Lastly, another study sampled various stomach contents of 8 estuarine and sea bird species that died or arrived dead in rehabilitation centres in southern Portugal. The authors found that 3 out of the 8 species (*Laurus fuscus, Laurus michahellis, Ciconia ciconia*) had plastic contents in their gastrointestinal tract. The White stork (*C. Ciconia)* was the species with the highest prevalence (FO: 43.4%, n=9). Overall, the number of plastics ranged from 0 to 5 pieces per individual. Crucial data like the size of the plastics found and total amount were lacking.

In coastal and estuarine systems Lourenço et al., (2017) examined faecal samples of different waterbirds (*Calidris alpina* n=39; *Calidris alba* n=59; *Chariadrius hiaticula* n=30; *Limosa limosa* n=32; *Recurviostra avosetta* n=5) across different estuaries with increasing anthropogenic influence (from high to low: Tejo, Banc d'Arguin and Bijagós estuaries). On the Tejo estuary, microplastic concentrations in the samples ranged from 2.29 (*L. limosa*) to 17.78 (*R. avosetta*) items per ml. Frequency of occurrence were also high ranging from 66% (*L.limosa*) to 83% (*C. alba*). Additionally, the frequency of occurrence in benthic macroinvertebrates in the Tejo estuary (that most waterbirds sampled feed on) ranged from 90% to 100%.

Plastic additive environmental and contamination studies in biota are fewer and scarcer than microplastic ingestion reports. The only, and probably first, scientific paper reporting additive contamination in waterbirds was that of Huber et al., (2015), which showed a broad cocktail of additives present in the eggs of 3 species of waterbirds (common eider - *Somateria mollisima*; European shag - *Phalacrocorax aristotelis*; European herring gull - *Larus argentatus*) in 2 different colonies. A large portion of the chemicals found were Polychlorinated Biphenyls (PCBs). Other additives were also detected, albeit in much lower proportions (PBDE, PAH and Phthalates).

1.5 - (Micro)plastic pollution along the Portuguese coast

Portuguese coastal systems are not an exception to the problematic of (micro)plastic pollution. Portugal's large coastal area and hydrodynamics make it prone to (micro)plastic accumulation. Portugal's underwater current system is supplied by two of the north Atlantic's intergyres (the North Atlantic current from North to South, and the Azores current to the south). These intergyres progressively bring marine debris from North America, through the North Atlantic and into Portugal's west and southern coast. Marine debris and other pollutants can then be entrapped in Portuguese coastal systems. Moreover, direct input from Portuguese rivers and coastal pressure originating from a higher affluence of people living in coastal cities, the mismanagement of fishing gear, litter, and landfills, as well as recreational activities and large volumes of tourism, also contribute to Portugal's susceptibility to this problem (Cole et al., 2011).

As of 2018, the annual production of plastic waste in Portugal was of 93 118 tons/year (INE, 2018). During 2016, Portugal produced approximately 270 687 tons of mismanaged waste, including 5 717 tons of mismanaged plastic (Prata et al., 2020), of which, an unknown quantity ended up in the environment. Data calculated by Prata et al. (2020) estimated that in 2018, 1.5 trillion pieces of microplastics were dumped into rivers through treated waste waters and 2.7 trillion pieces were dumped through untreated waste waters. Although wastewater treatment plants can retain most microplastic particles (83% to 99%) a percentage will still get through (Prata et al., 2018). Wastewater effluents (either treated or untreated) containing microplastics are more prevalent in the north and centre of Portugal, followed by the Algarve and Alentejo (south) regions (Fig. 2). Likewise mismanaged plastic is more common in the centre and north when compared to the south (Fig. 2). Additionally, microplastics that get removed in water treatment plants, ultimately accumulate in the sludge, which is then dumped on landfills, or used in agriculture as fertilizers. Microplastics can then be transferred to nearby rivers where they can eventually reach coastal systems (Prata et al., 2018).

Studies documenting concentrations of microplastics have also been conducted in some Portuguese rivers. Rodrigues et al., (2019b) reported that the concentration of microplastics on the surface of the Douro River (1-2m depth), a river with heavy anthropogenic influence from an adjacent metropolitan area, was of 17.1 pieces/m^3. In the Antuã river (Rodrigues et al., 2018), a river heavily influenced by industrial zones, concentrations of 58 to 1265 pieces/m3 in the water column were reported. Furthermore, a range of 18 to 629 microplastic per kg of sediment was found. Both concentrations varied spatially and temporally. Additionally, microplastics found were polymers of polyethylene, polypropylene, polystyrene, and polyethylene terephthalate. The authors concluded that higher amounts of microplastics in the river Antuã were found near the pollution sources (i.e industrial zones) although not exclusively as they can be transported downstream. Therefore, since Portugal's microplastic contamination sources (industry) are mainly inland, watercourses such as rivers end up being one of the main vectors for microplastic distribution and input into Portuguese coastal systems (Antunes et al., 2018).

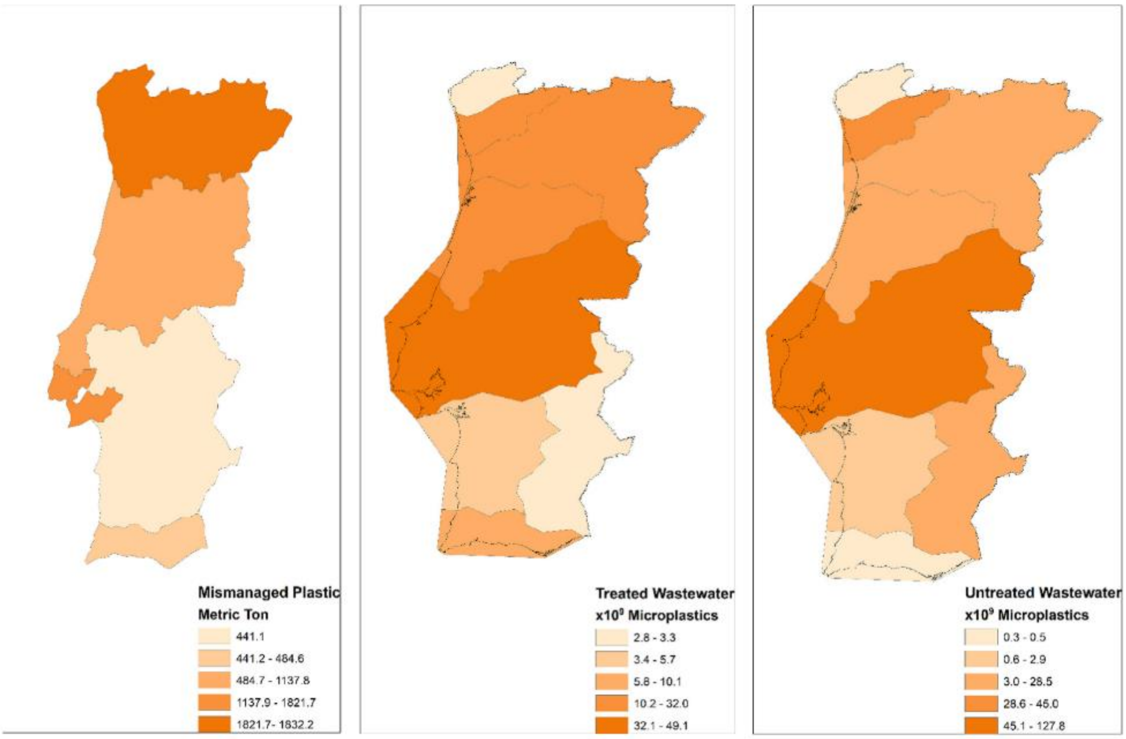

Figure 2 - Estimated quantities and sources of microplastic input into Portuguese environments. Mismanaged waste production in metric tons is represented on the left. Highest amounts of mismanaged plastic are produced in the North, Centre and Lisbon regions followed by Algarve and lastly Alentejo. Microplastic input into the environment through treated (centre) and untreated wastewater (right) effluents show highest amounts on the Tejo river basin. In Prata et al. (2020).

Coastal ecosystems are known to be zones of microplastic accumulation (Frias et al.,2014). In Portugal the range of sizes of plastics found on intertidal zones on beaches ranged from 50 µm to 20 cm. In total, 72% were microplastics and the biggest hotspots were in Lisbon and central Portugal (Peniche) (Martins et al., 2011). Antunes et al. (2013) evaluated the prevalence of microplastics in the form of resin regurgitates in several coastal regions of Portugal. The authors found a positive relation between the presence of harbours and industrials zones and the occurrence of a larger amount of resin regurgitates. In addition, they found that some contaminants were attached to the sampled regurgitates, including PAH, PCBs, and DDT (organic pollutants). The authors concluded that zones like the harbour of Sines constitute major hotspots for microplastic accumulation due to the high traffic of boats. Moreover, Candeias (2015) found that the north and centre of Portugal were the biggest hotspots for plastic accumulation, when compared to Lisbon, Alentejo and Algarve (by this order). The Algarve region had the lowest incidence of beached plastics, due to beach cleaning programs, geomorphology of the coast and its hydrodynamics. However, Frias et al. (2016) showed that in the Algarve region's coastal sediments, synthetic microfibers and fragments were found below 20 meters of depth and at the sediment level. The frequency of occurrence across 27 samples was of 56% with a total of 31 particles being found.

Portuguese estuaries are coastal systems also affected by microplastic accumulation. In the Tejo estuary, analysis of the sediment revealed high concentrations of fibres (7.5 pieces/ml) occurring in all 18 sediment samples (Lourenço et al., 2017). In the Douro estuary, microplastics of different colours and shapes were reported to being ingested by fish larva. A total of 2152 microplastic pieces in 1498 fish larva was reported. Fibres and fragment were the most prevalent type, thus indicating their prevalence in the Douro estuary (Rodrigues et al., 2018).

The aforementioned studies contribute to the establishment of baseline values for environmental surveys. These studies are then ultimately indispensable for microplastic and additive ingestion studies in species that inhabit and forage in these coastal environments.

1.6 - Objectives

As (micro)plastic pollution and subsequent ingestion by wildlife occurs at a worldwide scale, mediatic attention to this problem continues to grow. It is now almost impossible for the scientific community and the overall public to overlook it. This is reflected in the number of articles that have appeared in the last 20 years revolving around microplastics and their additives. Waterbirds and seabirds are particularly susceptible to this problem as they may potentially ingest large amounts of (micro)plastics and/or additives. Due to the intrinsic characteristics of their individuals and communities, waterbirds can hypothetically act as an early warning for additives and microplastic exposure and effects, thus, warning of a potentially dangerous contamination for the human and other species, with the potential of serving as an indispensable sentinel for the detection of microplastics and additive contamination. Yet, in order for them to be proven as useful sentinel species, a wholistic view with regards to waterbird species that occupy different ecological niches and that share common food sources with humans (crabs, mussels, fish etc…) should be taken into account. To date, no studies were found documenting (micro)plastic and additive uptake for many waterbird species likely to be exposed to high levels of these contaminants, making it impossible to relate microplastic and additive ingestion in species that forage in different, albeit adjacent areas, and that occupy different ecological niches - marine birds that forage the ocean and coastal regions, and coastal/shore birds that mainly forage in estuarine regions. Establishing a microplastic and additive consumption gradient from the estuary towards the sea using different species with different ecological niches could prove useful as it reveals a wholistic view of the problem across different areas, ultimately helping decide which species could be classified as sentinel species.

In this thesis we will: (1) describe microplastic and plastic additive contamination (BDE 28; 47; 99; 153; 154; 183) in six species: the Yellow-legged gull (*Larus michahellis*), the Black-winged stilt (*Himantopus himantopus*), the Pied avocet (*Recurviostra avossetta*), the Kentish plover (*Charadrius hiaticula*), the Little tern (*Sternula abifrons*) and the Audouin's gull (*Ichthyaetus audouinii*); using their faeces, regurgitates and eggs and following standardized descriptive methods; (2) verify if an hypothetical gradient of (micro)plastic contamination from the estuary towards the ocean exists; (3) Relate the amount of microplastics ingested with the amount of PBDE's present in the eggs of the species; (4) Compare the sensitivity of different types of samples (faeces and regurgitates) to reflect (micro)plastics exposure (5) Discuss if waterbird species can eventually be used as proxies for (micro)plastic and additive pollution and which of the sampled species might be potential candidates for monitoring plastic and additive trends. We, therefore, hypothesise that: (1) The ecological niche of each species and its feeding strategy will affect the quantity and type of (micro)plastics ingested and additives assimilated; (2) There will be a gradient of contamination from the species that forage in the estuary (where exposure is higher) towards the ocean foraging species (where exposure is lower). The Yellow-legged gull is expected to have the highest amounts and presence of plastics in the regurgitates and faeces (due to their interactions with landfills), followed by the Black-winged stilt, Pied avocet, Kentish plover, Little tern, and lastly the Audouin's gull; (3) Species that consume more microplastics will present a higher concentration of assimilated PBDEs in the eggs. (4) Regurgitate efficiency will influence the quantity and type of plastics found in the faeces and ultimately in the regurgitates.

Chapter II – Materials & Methods

2.1- Study species

In order to assess microplastics and additive exposure in waterbirds, adequate target species must be chosen. For the selection process, some pre-requisites should be considered. Species that are top predators that forage over a relatively large area, have a year-round population in the target area (optional but preferential), sensitive to the type of pollution intended to study, and have a geographical and foraging distribution over the study area should be considered for comparative purposes (Furness and Camphuysen, 1997; Avery-Gom et al., 2013). If the goal is to obtain a wholistic view of exposure, birds with different feeding strategies should also be considered (Avery-Gomm et al., 2013). Defining a target species (or group of species) is essential to compare results and obtain a spatial and temporal trend of contamination (Nicastro et al., 2018). Some waterbirds fulfil the pre-requisites mentioned above and therefore were chosen for this study. These species include the Black-winged stilt (*Himantopus himantopus*), the Kentish plover (*Charadrius hiaticula*), the Pied avocet (*Recurviostra avossetta*), Audouin's gull (*Ichthyaetus audouinii*) the Yellow-legged gull (*Larus michahellis*) and the Little tern (*Sternula abifrons*). They have relatively different diets, different foraging strategies, resident populations and different ecological niches that enable us to establish a gradient from the estuary towards the ocean.

2.1.1 - Black-winged stilt (*Himantopus himantopus* - HH)

The Black-winged stilt (Fig.3a) is a charadriiform belonging to the Recurvirostridae family. This species has a wide distribution, being found in almost every continent. They can be commonly spotted in fresh and brackish water wet zones such as marshes, estuaries, and salt pans where they form small groups (often mixed with avocets) to breed during the months of April through June in Portugal. Black-winged stilts mainly feed on insects and crustaceans often found at the surface of the water or at the sediment level (i.e bottom of a pond or in the sand). They mostly peck in order to catch already visible prey. However, several instances have been reported where they have been found to be probing the sediment tactilely for crustaceans, taking short jabs at the sediment (Goriup, 1982). After catching their prey, stilts are also known to wash their prey in the water.

2.1.2 - Pied avocet (*Recurvirostra avosetta* - RA)

The Pied avocet (Fig. 3b) is another charadriiform from the Recurviostridae family. Their distribution ranges from the Palearctic regions (where they reproduce) to south Africa. In southern Portugal, however, there are resident populations that stay all year round, usually inhabiting wetlands with fresh or brackish water. These areas include mud flats, salt pans and marshes. They feed mainly on insects, crustaceans, bivalves, and other macroinvertebrates found along the sediment. This species of avocet possesses an characteristic long thin beak curved upwards. To catch prey, they insert their beaks slightly open in the water or inside the sediment, and similar to a duck, shaking it rapidly sideways. This species uses their tactile abilities to distinguish between food and non-food items.

2.1.3 - Audouin's gull (*Ichthyaetus audouinii* - IA)

One of the members of the Laridae family that was sampled was the Audouin's gull (Fig.3c). Their distribution is mostly focused on the Mediterranean Sea with a resident population in southern Portugal and a relatively large colony in Deserta Island in Algarve (approximately 4245 reproducing pairs in 2021, personal observations). They form their colonies on sand dunes not far from the sea. Contrary to other gull species, the Audouin's gull does not scavenge (feed on leftovers), being strictly coastal or pelagic. Furthermore, they do not wander far from their colonies and mostly forage during the night. Their diet is comprised mostly of fish. They catch their prey by dipping (picking up prey at the surface with their beaks or fangs) or by diving.

2.1.4 - Yellow-legged gull (*Larus michahellis* - LM)

The Yellow gull is the other gull species used in this study (Fig. 3d). One of the most widely distributed species in the European continent, the Yellow-legged gull also has resident colony (which has been decreasing with around 591 reproducing pairs in 2021, personal obersvations) in southern Portugal in the Deserta Island in Algarve. They also build their colonies in sand dunes. Yellow-legged gulls have been known to take long foraging trips. This species is an omnivore, eating almost anything that resembles food. Their diet is comprised of fish, crustaceans, worms, eggs, and juveniles of other bird species. Additionally, Yellow-legged gulls have also been known to frequent landfills (Fig. 6b) where ingestion of anthropogenic items in large quantities have been reported (Lopes et al., 2021). When foraging for natural food items (mostly fish) they mostly resort to scavenging (eating leftovers), kleptoparasitism or dipping.

2.1.5 - Little tern (*Sternula albifrons* - SA)

The Little tern (Fig. 3e) is another member of the Laridae family that was also sampled for this thesis. In southern Portugal during the summer, small colonies of this species inhabit zones like sandy beaches and salt pans. Little terns usually forage around the vicinity of their nests, which means that depending on the location of the nest they might forage in the ocean or forage in the lagoon system of Ria Formosa. Their diet is mainly comprised of small fish, although sometimes crustaceans and insects can also be found. They catch their prey by surface plunging, diving (catching their prey at the surface with their beak – Fig. 3e) or by dipping.

2.1.6 - Kentish plover (*Charadrius alexandrinus* - CA)

The Kentish plover (Fig. 3f) is a waterbird belonging to the Charadriiform order. This small bird is widely distributed around the Mediterranean Sea with a resident population in the Algarve. These plovers inhabit zones such as saline lakes, lagoons, salt pans, dunes, and marshes. Their diet is known to be comprised mostly of terrestrial and aquatic insects, crustaceans, molluscs, and seaweed. This species forages both in marine and freshwater habitats, usually on rocks covered by water and in intertidal zones, often running after their prey and picking them up directly from the surface of the substrate with their beaks.

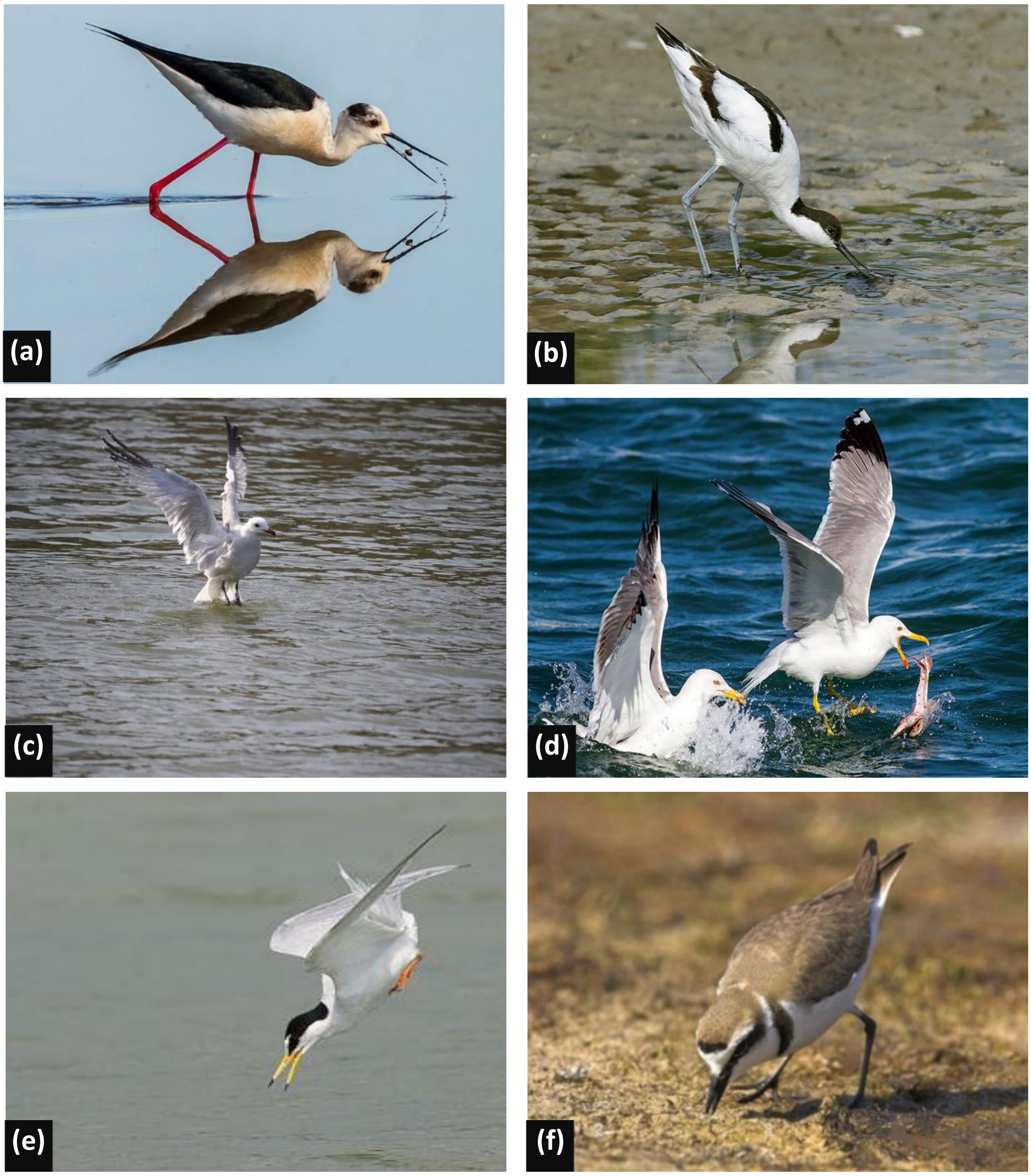

Figure 3 - Species sampled for this thesis: (**a**) Black-winged stilt foraging. Photo by Frank McClintock; (**b**) Pied avocet foraging along the sediment. Photo by Neokortex, 2010; (**c**) Audouin's gull catching prey by way of dipping; (**d**) Yellow-Legged gull feeding on dead prey; (**e**) Little tern diving after prey, photo by Lee Tiah Khee; (**f**) Kentish plover foraging along coastal sediment. Photo by Oliver Smart.

2.2 - Study area

Ria Formosa (36° 59' 31.46" N 7° 55' 21.90" O) located in-between the Ancão peninsula and Manta Rota in Algarve in the southern part of Portugal, is a mesotidal lacunar system with roughly the shape of a triangle encompassing a total area of 18 000 ha (Fig.4). This lagoon is bordered to the north, west and east by human settlements and to the south by barrier-islands which comprise the interface between the lagoon and the sea. To the North, five small freshwater courses drain into the lagoon (Ribeira de São Lourenço, Rio Seco, Ribeira de Bela-Mandil, Rio Gilão, Ribeira do Almagem). Yet, their freshwater input is almost negligible as salinity inside the lagoon stands stable and close to 36 ppt (Duarte et al., 2008).

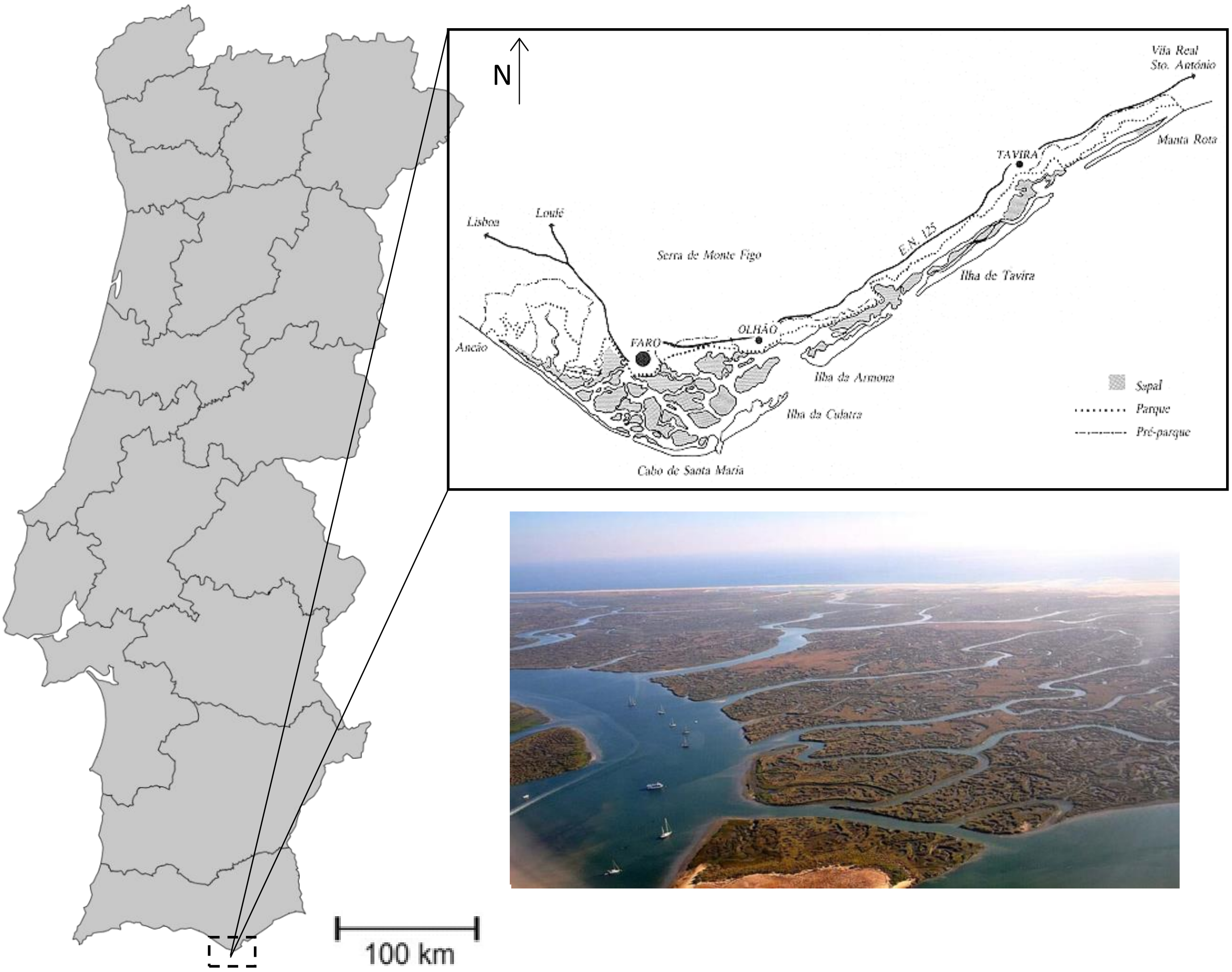

Figure 4 - Map of the location of the Ria Formosa lagoon in southern Portugal. Top image shows a map of the lagoon with the name of the barrier islands. Image bellow shows an aerial photo of a part of Ria Formosa during low tide.

Ria Formosa is widely known for its large diversity of fauna, flora, and habitats, ranging from barrier-islands to salt marshes, shoals, dunes, beaches, salt pans, and small lagoons of brackish or fresh water. It is also home to a large population of bivalves such as oysters and clams, being considered a natural nursery for these species. Hence, the region is known for the capture and aquaculture of in natura bivalves, limpets, cephalopods, and other seafood delicacies. Current data shows that nursing and catching of seafood represents around 90% of the total seafood consumed in the rest of the country (Silva & Cravo, 2020). Aquacultures, fishing, and salt production are the other main economic activities currently performed in the lagoon. Moreover, due to its sandy beaches on the barrier islands (mainly Deserta, Armona and Culatra island), the Ria itself acts as a tourist attraction, attracting many tourists every year during the summer.

The large availability of food resources and habitats offered by the Ria Formosa attracts a large number of different waterbirds which visit the lagoon or inhabit in it all year round. Due to its biological significance, Ria Formosa has been classified as a natural park and a special protection zone under European Union directives. It has also been ruled under the Ramsar convention for the protection of wetlands. Despite regulations, Ria Formosa still houses 10 wastewater treatment plants that often dump effluents directly into the lacunar system (Duarte, 2008).

2.3 - Field work

Sample collection was performed during the reproductive season of 2021 (between March and July). Nests (or colonies) were geolocated prior to sample collection. Several Pied avocets, Black-winged stilts, and some Little tern nests were located in saltpans, while nests of the Kentish plover, Little tern and both colonies of gulls were located in sand dunes. Sample collection was done under an ICNF license (Instituto de Conservação da Natureza e Florestas – the Portuguese government's environmental institute) and following all ethical guidelines to ensure that minimal levels of stress were induced to the birds.

Faeces collection was made opportunistically. Treks were taken to find the bird's faeces in the vicinity of their nests (Fig. 5a). Only fresh samples were collected to avoid

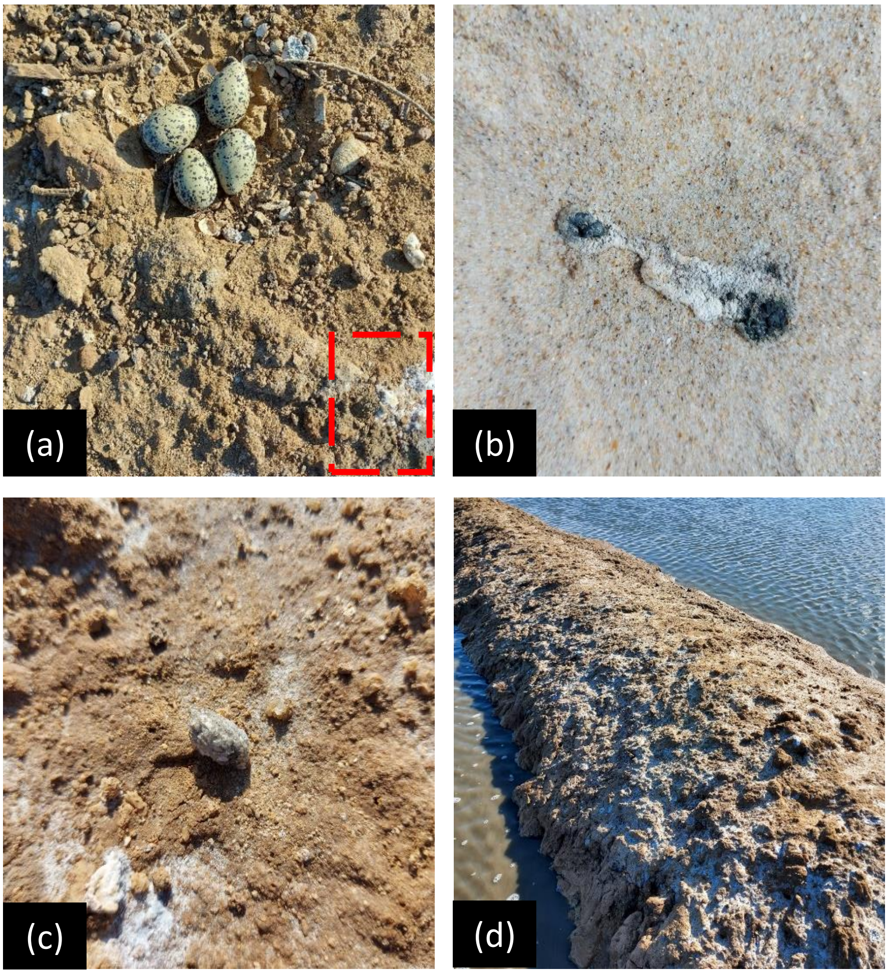

Figure 5 – (**a**) Pied Avocet nest with faecal matter close to it; (**b**) Fresh faecal sample of a Little tern; (**c**) A still intact Little tern pellet; (**d**) Resting spot in the salt pans in Tavira (Algarve).

any aerial contaminations possibly present in the area. Faeces were collected only if they were still wet (Fig. 5b). Given the high air temperature in the area during the sampling period, samples would dry quickly, therefore, collecting only fresh samples ensured us that the faeces were exposed to the surrounding air for a short period of time. All samples were collected with the help of a spatula (rinsed with ethanol between each use), into 5 ml plastic tubes and subsequently identified with location, species, date, identification number and stored in the cold until they could be stored in a freezer(-20ºC.). Since waterbirds sometimes defecate while in the nest incubating the eggs, we assumed that the faeces collected belonged to the species of each nest. Some samples were also collected opportunistically after the release of gulls that were captured to be ringed and processed as part of their long-term monitoring program. Both gull species would often defecate after take-off, probably due to the stress induced by handling. In other occasions, mostly for the waterbirds in the salt pans, the birds were often observed with binoculars until they defecated. The sample was then collected shortly after.

Regurgitates (Fig.5c) (of the Little tern, Audouin's gull and Yellow-legged gull) were mostly collected, in resting spots (Fig. 5d) and/or in the vicinity of their nests. Treks were taken along the Audouin's gull, Yellow-legged gull and Little tern colonies to find these samples. Regurgitates were collected using a spatula and stored in 1.5ml, 5ml and 50 ml plastic tubes or in small plastic bags, depending on the size of the sample. They were subsequently identified with location, date, species, ID number and stored in the cold before being frozen (-20ºC.).

Lastly, 10 eggs belonging to each sampled species were collected. Most of the collected eggs had been recently rejected or abandoned (given the high disturbance levels by humans and domestic animals in some areas). The eggs were stored in egg cartons in the cold before processing. Each egg was identified with location, date, and species.

2.4 - Laboratory work

Laboratory procedures for the faeces and regurgitates, followed an in-house made protocol, based on Bessa et al., 2019 (JPI Oceans) proposed best practices for microplastic studies. All procedures were conducted at room temperature in a room where all windows and doors were closed, in order to avoid any air current that would resuspend any microplastic particles (mainly microfibers) in the air. Before each working session, the benchtops where the work would take place and the acrylic chamber used to store the samples (see below) were rinsed with ethanol and left undisturbed for 30 minutes. This allowed any remaining suspended microfiber to deposit back into the tabletops therefore minimising airborne sample contamination. Additionally, the door leading into the room was slowly opened and closed each time someone needed to enter or exit the room. To eliminate any further contamination sources, nitrile gloves and cotton lab coats were also used. Surgical facial masks (which were mandatory due to COVID-19 preventive measures) used during sample manipulation were coloured pink. This served as a control for contamination since pink microplastics are not known to be prevalent in the environment. No pink microfibers were present in the controls nor in the samples (see control section in the results). Lastly, to assess the amount of aerial contamination inside the room, 2 control glassfibre filters (Branchia microfiber glass paper filter, grade BGF-3, 47mm) for each batch of processed samples (10-15 samples) were placed inside plastic

Petri dishes with the lid open. One control was placed in the benchtop where the work was being conducted and the other inside the acrylic chamber. Both controls were open while the procedures were taking place. Regurgitate processing only required a control on top of the benchtop and none inside the acrylic chamber since the procedures were done in only a couple of hours and therefore there was no need to store the samples inside the acrylic chamber.

PBDE quantification in the eggs was performed using an in-house made protocol. This part of the work was developed in the facilities of the Faculty of Pharmacy of Porto. Detailed procedures are also shown further.

2.4.1 - Faeces processing

In total 469 faecal samples were collected in the field. However, due to time constraints, 31 to 37 samples were randomly selected for each species (except for the Black-winged stilt, from which all 8 samples collected in the field were processed). Overall, 173 samples were processed. The samples were thawed and subsequently dried in an oven at 40ºC (covered with tinfoil to avoid contaminations inside the oven). They were then weighed in a balance to the nearest 0.00001g to obtain their dry weight and stored at room temperature before being processed.

The first phase of processing involved digesting the samples to remove faecal matter. To achieve this, KOH at 10% was prepared in a hotte using KOH flakes (Potassium hydroxide 85%, LABKEM) and distilled water. The mix was then stored in a 5L glass bottle. Before each usage, the mixture was filtered using a vacuum pump with a glassfibre filter (Branchia microfiber glass paper filter, grade BGF-3, 47mm) (as shown in Fig. 6) and stored again in a closed glass bottle. The samples were then re-hydrated with filtered distilled water (4 ml per sample) and homogenized with the help of a needle (rinsed with ethanol at 70% between each usage) and/or using a vortex to displace the faecal matter. Afterwards, the displaced samples were transferred to 30 ml glass jars and covered with the lid of a glass Petri dish. All glass jars and glass Petri dishes were previously washed using tap water, rinsed with ethanol at 70% and placed in an oven until dry. The displaced samples inside jars were then diluted with KOH at 10%, using a 1:3 volume ratio (sample:KOH). The glass jars would then be stored inside the acrylic chamber for 18 to 20h, (max. 48h) to let the faecal matter dissolve. To facilitate the digestion process, lumps of faecal matter were fragmented using an ethanol rinsed needle.

Figure 6 - Setup of the vacuum pump kit used to filter the samples. Column was always covered with a glass Petri dish to avoid any unwanted airborne contaminations of the sample filters. Control filter is opened near the working area shown (in the background).

After digestion, the resulting liquid was filtered using a vacuum pump (Fig.6). To filter the samples, glassfibre filters (Branchia microfiber glass paper filter, grade BGF-3, 47mm – mesh size 1µm) were used. Once in place, a claw was used to attach the column into the rest of the vacuum pump system. During the filtering process, the column containing the sample was covered with a glass Petri dish lid to avoid the suction of any airborne particles into the filter (Fig. 6). Between each sample the column and the Petri dish lid were washed using a jet of tap water and rinsed with ethanol and distilled filtered water (Fig 7 a-c). The column was always covered with the Petri dish lid after washing as to avoid any aerial contaminations when moving it back to the benchtop (Fig. 7c). After filtration, the filters containing the samples were placed inside glass Petri dishes and put in an oven at 40ºC. for up to two days or until completely dried.

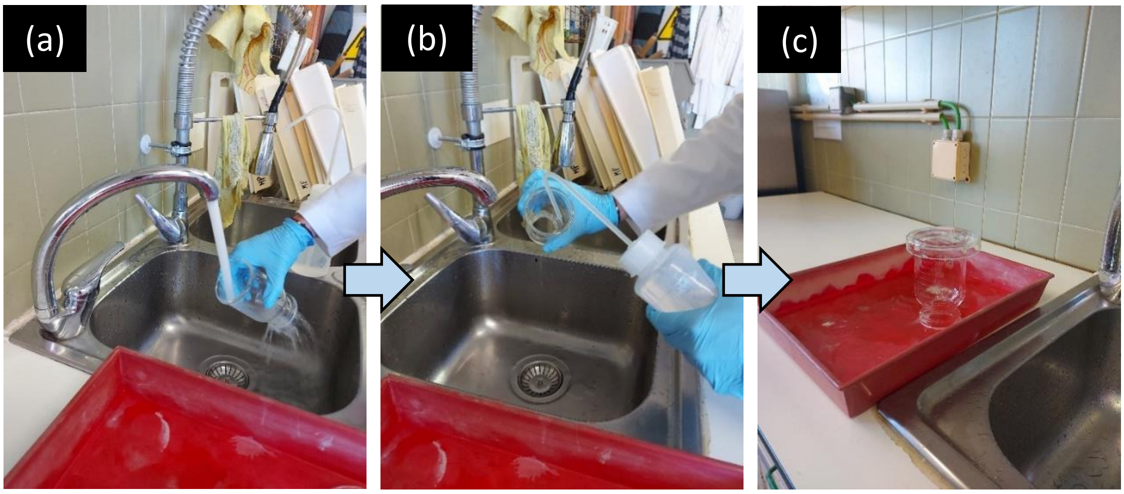

Figure 7 - Washing process of the vacuum pump's column, performed between filtering each sample: (**a**) The column was first washed with a jet of tap water; (**b**) Rinsed with ethanol at 70%; (**c**) Covered with the glass lid and transferred back to the benchtop to be directly reattached to the rest of the vacuum pump setup. The red tray shown was also frequently washed.

2.4.2 - Regurgitate processing

Similar to the faecal samples, 73 Little tern regurgitates were randomly chosen out of 128 regurgitates. All 59 Audouin's gull and 77 Yellow-legged gull regurgitates were processed. All regurgitates were thawed and put in an oven (while covered in tinfoil) to dry at 40ºC. for up to 2 days.

For the regurgitates of both gull species, a sorting of plastic materials and fish leftovers was performed using a stereomicroscope. Particles that resembled plastics were sorted and stored. Afterwards, each (micro)plastic was measured using a stereomicroscope and millimetric paper. Tweezers were used to separate and unravel entangled plastic fibres and films. Additionally, the type and quantity in each sample was also registered.

Little tern regurgitates were processed using a differential density separation by way of a solution of NaCl with an approximate concentration of 200 mg/ml. This solution was made using laboratory grade sodium chloride crystals and distilled water and was subsequently stored in a glass jar covered with a glass lid. After preparation, the solution was then filtered in a vacuum pump with a glass fibre filter and stored again in another covered glass jar. The regurgitates were then removed from their respective casings and placed inside 30 ml glass jars covered with glass Petri dishes. The NaCl solution was added to the regurgitates in a proportion of 1:3 volume (sample:NaCl). The samples were then left for 2 hours. Afterwards, the supernatant of the samples was filtered using a vacuum pump and glass fibre filters (same procedures as in the faeces). Leftovers from the regurgitates (mostly fish parts) were left at the bottom of the jars to later be discarded. The glass fibre filters were then placed inside enclosed glass Petri dishes and dried at 40ºC in an oven.

2.5 - (Micro)plastic detection and characterization

Once dried, the filters containing the filtered faecal samples and regurgitates were transferred from glass to plastic Petri dishes. The filters were then observed under a stereomicroscope using 30x and 40x magnification. All suspected (micro)plastics found, were transferred, and imbued (using a pair of tweezers) into grided 5x4 glassfibre filters inside plastic Petri dishes. Afterwards, the closed Petri dishes were securely taken into the MAREFOZ facilities (Marine and Environmental Sciences Centre – Figueira da Foz) where a camera coupled to a stereomicroscope (LEICA M60) was used to take pictures of each (micro)plastic. Each plastic was also measured using the software of the coupled camera (LAS version 1.4.8). Fibres were measured from end to end, while in fragments and other pieces the diameter was measured. Each plastic was then categorized into the following size classes: 5mm<Mesoplastic (Meso)<25mm; 1mm<Large microplastic (LMP)<5mm; 1µm<Small microplastic (SMP) <1mm; 1nm<Nanoplastic (NP)<1µm. Plastic type categorization was made according to Bessa et al., (2019).

Since the use of µ-FTIR to analyse and confirm the synthetic polymers of the presumed plastics was not a possibility due to time constraints, working criteria had to be created to distinguish between probable synthetic and natural particles, as well as discern between aerial contamination and natural occurring particles. Particles that did not fit the criteria were disregarded and not introduced into the grided filters. The criteria used are described as follows: (1) If the particle was not imbued in the filter or stuck to organic matter (Fig. 8c and 8d by contrast) indicated that the particle did not go through the digestive process and filtering and was a result of aerial deposition on the sample; (2) (micro)plastics that had a solid colour were likely to be contaminations (Fig. 8d) as particles that were ingested generally loose some colour due to the digestion process of the bird or the KOH (Fig. 8a, 8b); (3) The occurrence of a certain particle (same colour size and type) across different samples of different species was also used as a criterion to discard certain particles as contaminations (Fig.8e and 8f); (4) The occurrence of particles in the samples, identical to those in the control filters were labelled as contaminations and therefore also disregarded; (5) Presumed microplastics that appeared to be shiny (Fig.8c, d) were likely to be synthetic as shininess is not common to occur naturally (natural fibres - Fig.8e,f); (6) If a particle was durable (resisted when slightly stretched or pressed), it was likely synthetic, since synthetic particles tend to be more resistant than natural occurring particles; (7) Fibres that appeared to be less rugged were probable to be natural occurrences as synthetic fibres tend to be rugged due to the heavy processing that they are subjected to (Fig. 8e and 8f).

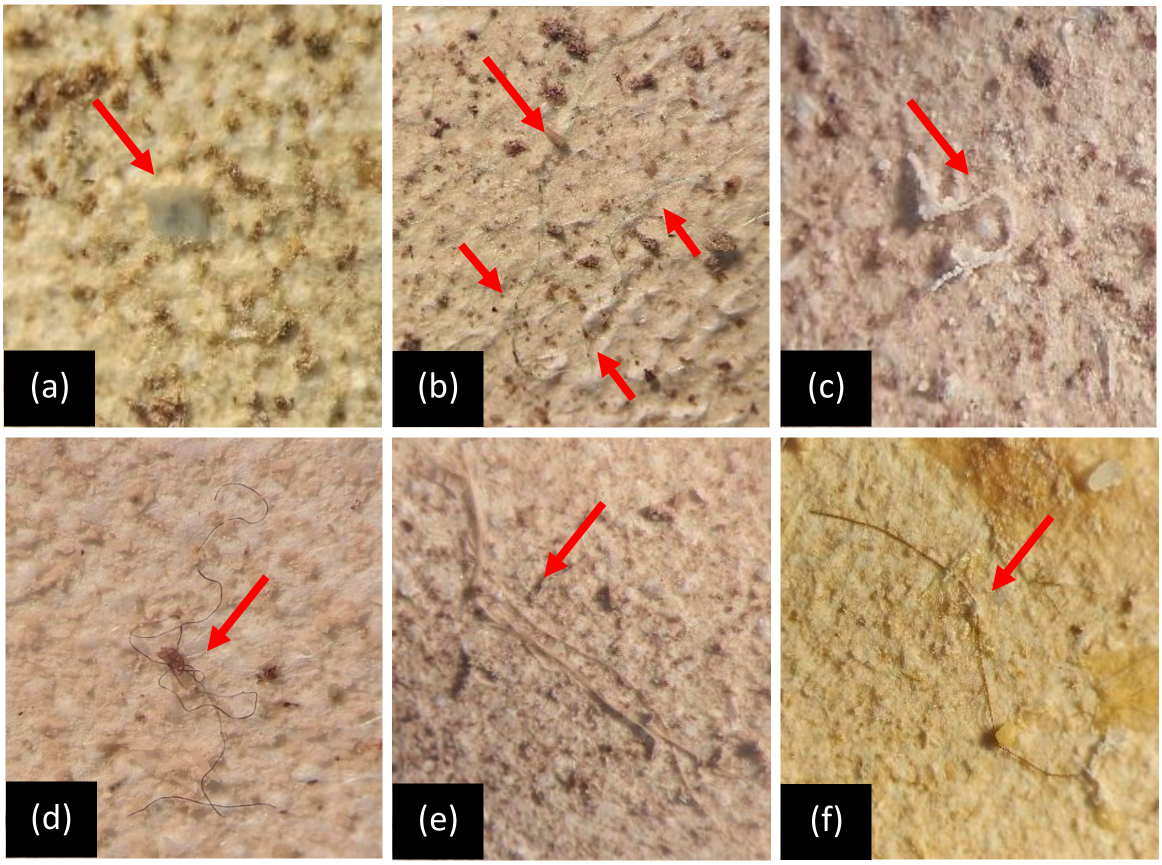

Figure 8 - (**a**) Blue fragment classified as an ingested plastic; (**b**) Battered blue fibres likely due to the digestive processes of the bird; (**c**) White fibre covered in organic matter; (d) Black fibres formed in a ball, not attached to the filter, thus considered a probable airborne contamination; (**e**) Natural fibres, probably plant matter; (**f**) Brown (presumed) natural fibre easily mistakable as a synthetic particle. This type of particle was prevalent among most samples.

These criteria were applied consistently during the selection process under the stereomicroscope. This allowed us to effectively determine what particles were aerial contaminations by cross-examining the particles present in the control filters and those in the samples. Furthermore, it enabled us to discern some natural occurring particles from synthetic microplastics. Yet, discerning natural from synthetic was considerably harder for some samples which had large amounts of faecal, plant and/or insect matter. These samples had large amounts of natural fibres (Fig. 9 a, b, c) that could easily be confused as synthetic particles.

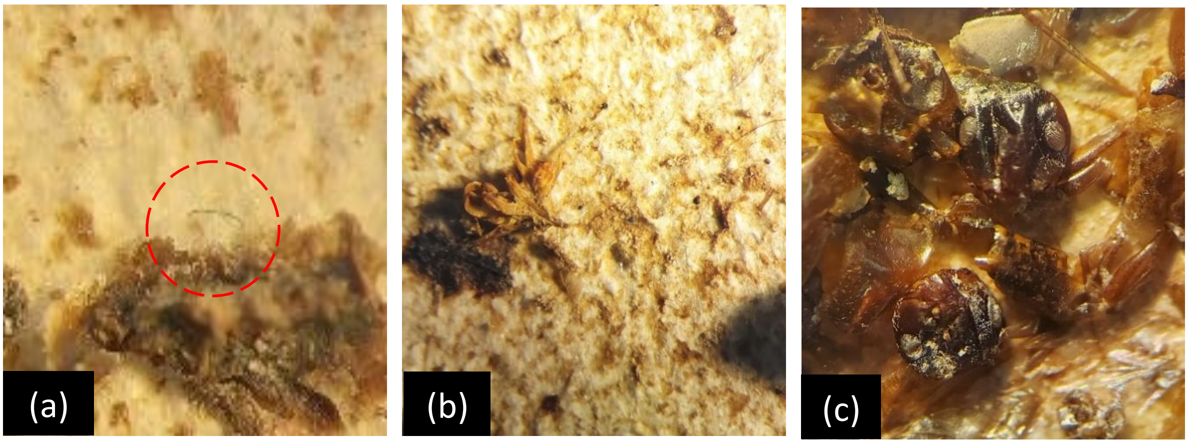

Figure 9 - (**a**) Blue fibre stuck in faecal matter; (**b**) Parts of a plant; (**c**) Chitinous carapaces belonging to insects.

2.6 - PBDE analysis in eggs

Egg processing and instrumental analysis were performed following an in-house protocol based on the methods used in Fangstrom et al., 2005; Karlsson et al., 2006; Van den steen et al., 2011; Bouwman et al., 2012 and Lu et al., 2019.

Firstly, each egg sample was homogenized using a blender (washed between each sample). The homogenates were then dried, identified, and separated in 500mg samples. To the samples, 2,5 ml of a mix of acetonitrile (CH_3CN) and toluene (C_7H_8) in a 4:1 ratio (v/v) was added. Next, 2 ml of distilled water were added. The samples were then left in a magnetic mixer overnight. The next day, all samples were vortexed, mixed with 1g of magnesium sulphate ($MgSO_4$) and 0.25g of sodium chloride (NaCl) and promptly vortexed again, as to not let the mixture harden. The mixture was then put on a centrifuge at 2500 RPM for 5 minutes. Afterwards, the supernatant was removed and placed in Amber vials. The supernatant was then spiked with 15µl of internal standard. Later, the samples were divided into fractions A and B, so that one was spiked with an external standard (BDE 37, BDE 77, 6MeO-BDE-47, or FBDE 126). To remove lipids and other substances that could cause noise while performing the Gas Chromatography-mass spectrometry (GC-MS), an enhanced matrix removal (EMR) kit was used (Bond Elut EMR-Lipid, Agilent). To activate the EMR, 1ml of distilled water was added to centrifuge tubes and vortexed. Then, 200mg of the activated EMR liquid were mixed with the fraction A of each sample and later centrifuged at 5000 rpm for 5 minutes. The supernatant of each sample was then transferred to 50ml centrifuge tubes and mixed in a vortex with 3,5g of the lipid $MgSO_4$ polish pouch (part of the EMR kit). The mixture was again centrifuged at 5000 rpm for 5 minutes and the supernatant transferred to Amber vials. The Amber vials were then put on a low temperature oven until all the acetonitrile-toluene mix (added previously) had evaporated. Next, 0,5mL of n-hexane and 0.25mL of sulfuric acid (H_2SO_4) were added to the samples and mixed in centrifuge tubes using a vortex. Afterwards, the samples were centrifuged at 2500 rpm for 5 minutes. The supernatant was put on 15ml centrifuge tubes where 0,5 mL of water were added, and the mixture was centrifuged again at 2500 rpm for 5 minutes. The supernatant was then passed through a column containing 200mg of neutral alumina, previously activated with 0,5ml of n-hexane. The precipitate was then eluted with 0,5 ml of n-hexane, dried in an oven, resuspended with 70 µl of trichloroethylene (C_2HCl_3) before being vortexed and centrifuged at 2500 rpm for 5 minutes. Lastly 1µl of the supernatant was introduced into the GC-MS machine.

An Agilent 6890 GC with an autoloader connected to an Agilent 5973 MS was used to perform the GC-MS analysis. Detection was made in negative chemical ionization (NCI) mode with methane as the reagent gas. A Zebron™ ZB-5HT w/GUARDIAN™ column (5 m, GC Cap. Column 30 m x 0.25 mm x 0.25 µm) was used. The mass spectrometer was set for single ion monitoring mode using the bromide ion isotopes m/z 79 and 81. Limits of detection were defined as three times the noise level. An eight-point linear calibration curve was used, and calculations were done within the linear range.

2.7 - Statistical Analysis

Statistical analyses were performed based in 2 types of data matrices (Table S1-S4 - supplement). Firstly, a binomial matrix was built (Table S1-S2 - Supplement) where the type, colour, and size class present were noted according to their presence (1) or absence (0) in each individual sample. Secondly, a data matrix was built with the number of plastics found of each category per sample (Table S3-S4 - Supplement). Categorization of each plastic was performed following the classification methods proposed by Bessa et al., 2019 (JPI Oceans). Please note that, plastics that were within the mesoplastic size range, due to their small amounts, were also considered for the subsequent statistical analysis. Data matrices with the size and number of plastics per sample and per sample weight were also built (Table S5-S7 - Supplement). Lastly, a matrix with the concentration (ng/500mg of egg sample) of additives (BDE 28, 47, 99, 154, 153, 183) per egg sample was also built (Table S8 - Supplement).

Normal distribution of the total number of plastics in each sample (in faecas and regurgitates) was not obtainable even after $\log_{10}$, square root and lognormal transformations. (Shapiro-wilk tests $p<0.05$).

The mean and standard deviation (SD) of the total number of plastics per positive samples (regurgitate or faecal samples with at least 1 plastic); size (mm); the total amount in faeces and regurgitates (number of plastics across all samples), were calculated and separate tables for each sample type were built (results).

Frequency of occurrence values were calculated using the binomial matrix. The formula used is listed below:

$$FO = \left(\frac{na}{ntotal}\right) \times 100$$

where "FO" is the frequency of occurrence, "na" the number of affected samples of a given category (i.e Fibre, fragment, blue) and the "ntotal" the total number of samples of a given species.

Since the FO bar plots were performed using cumulative percentages that reached above 100%, a formula adjusting those percentages to 100% was used:

$$FOa_{100} = \frac{Perc \times 100}{Perc_{total\ FO}}$$

Where "FOa_{100}" represents the adjusted FO to 100%, the "Perc" the FO of a given category and the "$Perc_{total\ FO}$" the sum of the overall FO of a group of categories of a given species (i.e sum of FO of types of plastics in the Yellow-legged gull).

Relative proportions of plastic categories found were calculated using the following formula:

$$Fq = \left(\frac{nq}{nqTotal}\right) \times 100$$

where “Fq” is the percentage of microplastics of a given category, “nq” number of microplastics of a given category and “nqTotal”, the total amount of microplastics on a given species.

Non-metric multidimensional scaling (NMDS) using the total amount of microplastics per sample found in each category was used to graphically represent the dissimilarities between the species. Only positive samples were considered for this analysis. Separate NMDSs were built for each group of categories (types, colours, and size classes) in both types of samples (faeces and regurgitates). Stressplots are shown in the supplemental data (Fig.S1-S6 - Supplement). Furthermore, a principal component analysis (PCA) using the data matrix of additive concentrations per egg sample (Table S8 – Supplement), was performed. A biplot was built with the resulting data, in order to analyse possible segregations between the groups (different species) and the influence of each additive in said dissimilarities.

To assess possible correlations between variables, Spearman’s rank correlation coefficients were calculated. Six-point correlations were tested between: (1) mean size of plastics in faecal samples and mean size of each species (g); (2) weight of the faecal samples (g) and the number of plastics present; (3) number of plastics per species and the mean size of each species (g); (4) PCA mean scores of each species on the first axis and the number of plastics consumed by each species. Additionally, a multi-point correlation between the number of plastics per sample and the weight of the corresponding faecal sample was tested. No correlations were performed for the regurgitates as a 3-point correlation would not comprise a robust analysis. Lack of weight data for all regurgitates further inhibited similar analysis. Mean body mass of the birds were extracted from: Audouin’s gull - Burger et al., 2020; Yellow-legged gull - del Hoyo et al., 2020; Black-winged stilt - Pierce et al., 2020; Kentish plover- del Hoyo et al., 2021; Pied avocet – Pierce et al., 2020b; Little tern - Gochfeld et al., 2020.

To assess the effect of the explanatory variable “Species” on each variable, generalized linear models (GLM) with different distributions and fitting models were used. It is important to note that although, the data matrixes included all variables, only those with an overall occurrence above 10% were used for the subsequent statistical analysis. Thus, variables “Total”, “Presence” (binomial matrix), “Fibre”, “Fragment”, “Blue”, “Transparent”, “Brown” and “Red” in the faecal samples and “Total”, “Presence” (binomial matrix), “Fibre”, “Blue” in the regurgitates were the variables tested. GLMs with a binomial fitting and logit distribution were performed for each of the variables mentioned above (except for variable “total” which was swapped for variable “Presence” in the binomial analysis). Additionally, GLM’s with a Poisson fitting and log distribution were also performed. To check for zero inflation and over dispersion, a ratio between the number of predicted and observed zeros was calculated (Table S9-S12 – Supplementary material). To account for a probable zero inflation, GLM’s with a zero inflated fitting were done. Lastly, to account for additional over dispersion, GLM’s with a zero inflated fitting and negative binomial distribution were performed. In order to check which model best suited the data, Akaike information criterion (AIC) and log-likelihood values were calculated and compared. Models which presented the lowest AIC and log values were selected for the analysis. Every GLM model used (and their results), their AIC, log values and zero ratios is shown in the supplement (Table S9-S12 – Supplementary material). For the comparison of sizes between species an GLM with Gaussian modelling and “Identity” distribution was used.

Frequency of occurrence and percentage of microplastics found were calculated using Excel 2019 (Microsoft Corporation). Scatterplots, graphics, and tables were also built in Excel. Non-parametric tests (Spearman’s rank correlation coefficients) were performed in STATISTICA version 12 (Statsoft, 2013). PCA, NMDS’s and GLM models were performed in R-4.2.0 (R Core Team, 2022) through the interface RStudio-2022.02.1 Build 461 (RStudio, PBC, 2022). Normality of data was checked using packages “car” (Fox & Weisberg, 2019), “nortest” (Gross & Ligges, 2015) and “moments” (Komsta & Novomestk, 2022) in R. The PCA was performed and ploted using packages “factoextra” (Kassambara & Mundt, 2020), “FactoMineR” (Lê & Husson, 2008) and “RColorBrewer” (Neuwirth, 2022). NMDS were done using the packages “Vegan” (Oksanen et al., 2022), “lattice” (Sarkar., 2008), “permute” (Simpson et al., 2022) and “dplyr” (Wickham et al., 2022). Lastly, GLM’s analyses were done using the base statistic package of R (R Core Team, 2022), “performance” (Lüdecke D et al., 2021) and “pscl” (Zeileis et al., 2008; Jackamn, 2020;).

Chapter III – Results

Table 1 - Plastics detected in faecal samples of waterbirds in Ria Formosa, Portugal. Total number of faecal samples analysed, number of plastics detected, plastics' frequency of occurrence (%), mean number of plastics (±SD) in the affected samples, mean size (±SD) and overall mean number (±SD) (all samples) per species.

Species	Total n (samples)	Occurence	Total n (micro)plastics	Mean number of plastics per affected sample (±SD)	Mean size (mm) (±SD)	Total mean ammount (±SD)
Black-winged stilt (HH)	8	75.00	18	3 ± 2	2.13 ± 1.83	2.25 ± 2.16
Pied avocet (RA)	33	60.60	62	3.10 ± 2.99	1.44 ± 1.27	1.87 ± 2.78
Yellow-legged gull (LM)	32	68.75	133	6.04 ± 4.61	2.09 ± 2.02	4.15 ± 4.74
Audouin's gull (IA)	32	62.50	39	1.95 ± 1.24	1.77 ± 1.20	1.21 ± 1.36
Kentish plover (CA)	37	51.35	38	2 ± 1.45	1.74 ± 1.63	1.02 ± 1.44
Little tern (SA)	31	38.71	17	1.41 ± 0.86	1.28 ± 1.18	0.50 ± 0.87
Overall	173	57.20	307	3.10 ± 3.22	1.83 ± 1.72	1.77 ± 2.87

3.1 - Controls

Filters used as controls revealed a low contamination rate. In total, across the 24 filters used during the processing of the faecal samples (12 inside the acrylic chamber + 12 outside), 48 fibres were found with a mean of 2 particles/filter (1.5 particles/filter inside and 2.5 particles/filter outside). The filters were exposed for an approximate period of 69 hours. The deposition rate was approximately 0.43 particles/hour for the area around the workbench and of 0.26 particles/hour inside the acrylic chamber. The acrylic chamber offers some protection against airborne contaminations, therefore, the filters placed inside the chamber were not as exposed to airborne contamination. For the controls used during the regurgitate processing, 10 microfibres were detected. A mean of 1 particle/filter was found. In this case the filters were only uncovered for approximately 10.5 hours, indicating a deposition rate around the workbench of 0.095 particles/hour.

3.2 - Faeces

In total, out of 173 faecal samples, 99 had at least one microplastic. This accounts for 57.20% of the samples (FO). Overall, 307 plastics (293 microplastics + 14 mesoplastics) were found with a mean of 3.10 ± 3.22 per faecal sample (Table 1). The average total amount (mean number of plastics across the contaminated and non-contaminated samples) was of 1.77 ± 2.87 microplastics per sample. Microplastic mean length was of 1.83 ± 1.72 mm (Table 1). Notably, the Yellow-legged gull showed the highest mean number of plastics per positive sample (6.04 ±4.61, n=133 plastics) and the highest overall mean load (4.15 ±4.74) (Table 1). The Black-winged stilt (HH) showed the highest frequency of occurrence (75.00%, n=8) followed by the Yellow-legged gull (LM) (68.8%, n=32) (Table 1, Fig.10a). GLM using a binomial fitting (using data of absence/presence), showed no significant differences in the frequency of occurrence of plastics among species (Table 2). GLMs using zero-inflated models with negative binomial distribution showed a significant influence of the variable “species” ($p<0.05$) on the total number of plastics per species. Statistical differences were noted exclusively in the Yellow-legged gull (LM) (reference species: CA) (Table 3).

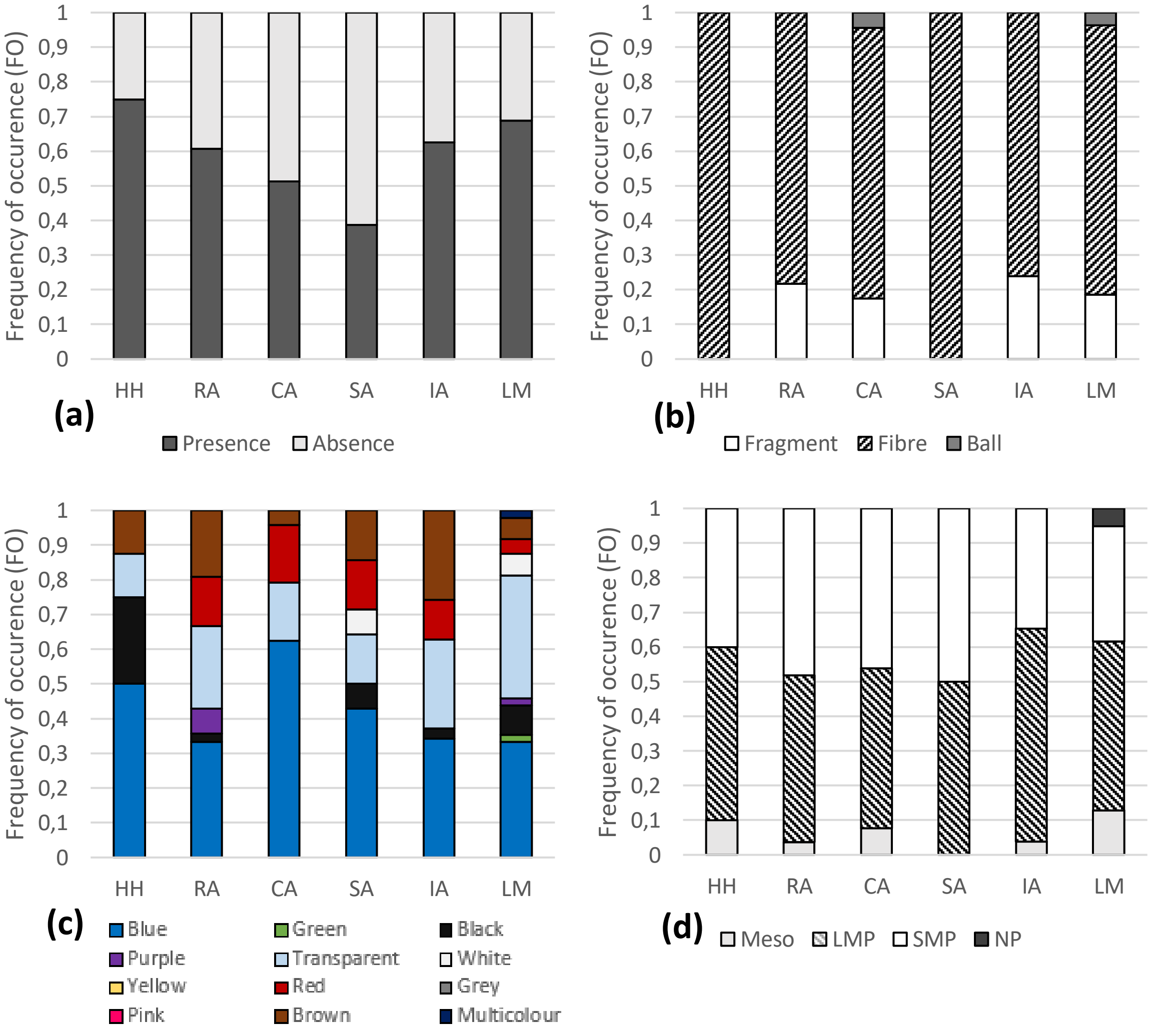

Figure 10 – Bar plots showing the Frequency of occurrence (FO) of all categories of plastics for each species’ faecal samples. Values adjusted to 1 (100%). Formula used can be found in the statistical methods. (**a**) FO of samples with at least one plastic (FO); (**b**) FO of the types of plastics in each species; (**c**) FO of the different plastic colours; (**d**) FO of plastic size classes (5mm<Meso<25mm; 1mm<LMP<5mm; 1µm<SMP<1mm; 1nm<NP<1µm). Species presented are listed as follows: HH – Black-winged stilt, RA – Pied avocet, CA – Kentish plover, SA – Little tern, IA – Audouin’s gull, LM – Yellow-legged gull.

3.2.1 - Plastic characteristics in faeces

Type

The most prevalent type of plastic found in the faeces were fibres (FO=54.34% - Table 5, Fig. 16b). Overall, 268 fibres were found. Additionally, fibres were prevalent across all species. Fragments were the second most common plastic type, found in 11.56% of the sample (Table 5, Fig. 16b). In total 37 fragments were found. Fragments were only present in 4 out of 6 species. Contrastingly, the rarest type was “balls of fibres” with 1.16% (Table 5, Fig. 16b). No filaments, films nor spheres were found. The GLM’s using the binomial model showed no significant differences in the presence of fibres among species (Table 2). Concerning the presence of fragments, only the Kentish plover (CA) samples revealed a significantly less presence ($p<0.05$), when compared to the other species (Table 2).

The Yellow-legged gull (LM)s had the highest diversity of plastics types (3 out of 6 types) whereas the Black-winged stilt (HH) had the lowest (only fibres). The Yellow-legged gull also showed the highest number of fibres (n=112), followed by the Pied avocet (RA) (n=55), the Audouin’s gull (IA) (n=34), Kentish plover (n=32), Black-winged stilt (n=18) and the Little tern (SA) (n=17) (Table S13- Supplementary material). Fragments were found in higher quantities in the Yellow-legged gull (n=20), followed by the Pied avocet (n=7), Audouin’s gull (n=5) and the Kentish plover (n=5) (Table S13- Supplement). Fibre proportions ranged from 100% in the Black-winged stilt and Little tern, to 84.20% in the Yellow-legged gull and Kentish plover. Fragments ranged from 14.70% in the Yellow-legged gull to 11.50% in the Pied avocet (Table 6, Fig. 11).

When comparing the effect of the species on the number of fibres, analysis of the GLMs using the zero inflated model revealed significant differences ($p<0.05$) among species, for the Black-winged stilt, Yellow-legged gull, and Pied avocet (Table 3). These species had a significantly higher number of fibres in their samples when compared to the rest of the species (Table 3). The Yellow-legged gull and the Pied avocet had the highest amounts of fibres (n=112 and n=55 respectively), and the Black-winged stilt one of the highest number of fibres per sample (Table S13 – Supplementary material). When comparing the number of plastic fragments in the faeces, only the Yellow-legged gull (LM) revealed significantly higher numbers than the rest of the species (Table 3). NMDS analysis (Fig. 12a) revealed no separation among species. Centroids were closely positioned along the axes. All species were closely associated with fibres. The only exception were samples from the Kentish plover and some from the Yellow-legged gull that were associated with fragments.

Colour

The colour “blue” was the most prevalent across all species with an overall frequency of occurrence of 38.73%. “Transparent” was the second highest occurring colour in 24.86% of the samples (Table 5, Fig. 16c), followed by the colour brown (13.87%) and red (10.40%). The least frequent colours were green and multicolour. No yellow, grey, or pink particles were found. GLM Binomial models using presence and absence of colours in the samples (Table 2), showed no significant differences (when compared to the reference species) for the colour blue. By contrast, significant differences were found among the Yellow-legged gull, and Pied avocet for the colour transparent. In the Yellow-legged gull and Pied avocet, “transparent” coloured plastics occurred in a significantly higher number of samples (more prevalent) (Table 2). Lastly, significant differences were noted for the Audouin’s gull and the Pied avocet when analysing the colour “brown”.

Out of the 307 plastics retrieved, a large portion were blue (n=125) and transparent (n=115) (Table S13- Supplement). The colours red (n=20) and brown (n=24) were also found, albeit in lower quantities. When comparing bird species, the Black-winged stilt (HH) had the highest proportion of blue plastics (77.78%, n=14), the Yellow-legged gull (LM) had the highest proportion of transparent plastics (57.14%, n=76), and a significantly higher quantity (Table 3) of transparent plastics. Likewise, the Pied avocet and the Little tern revealed a significantly higher quantity of “transparent” (micro)plastics when compared to the reference species (Table 3). Additionally, the Audouin’s gull showed a significantly higher amounts of “brown” (micro)plastics (Table 3).

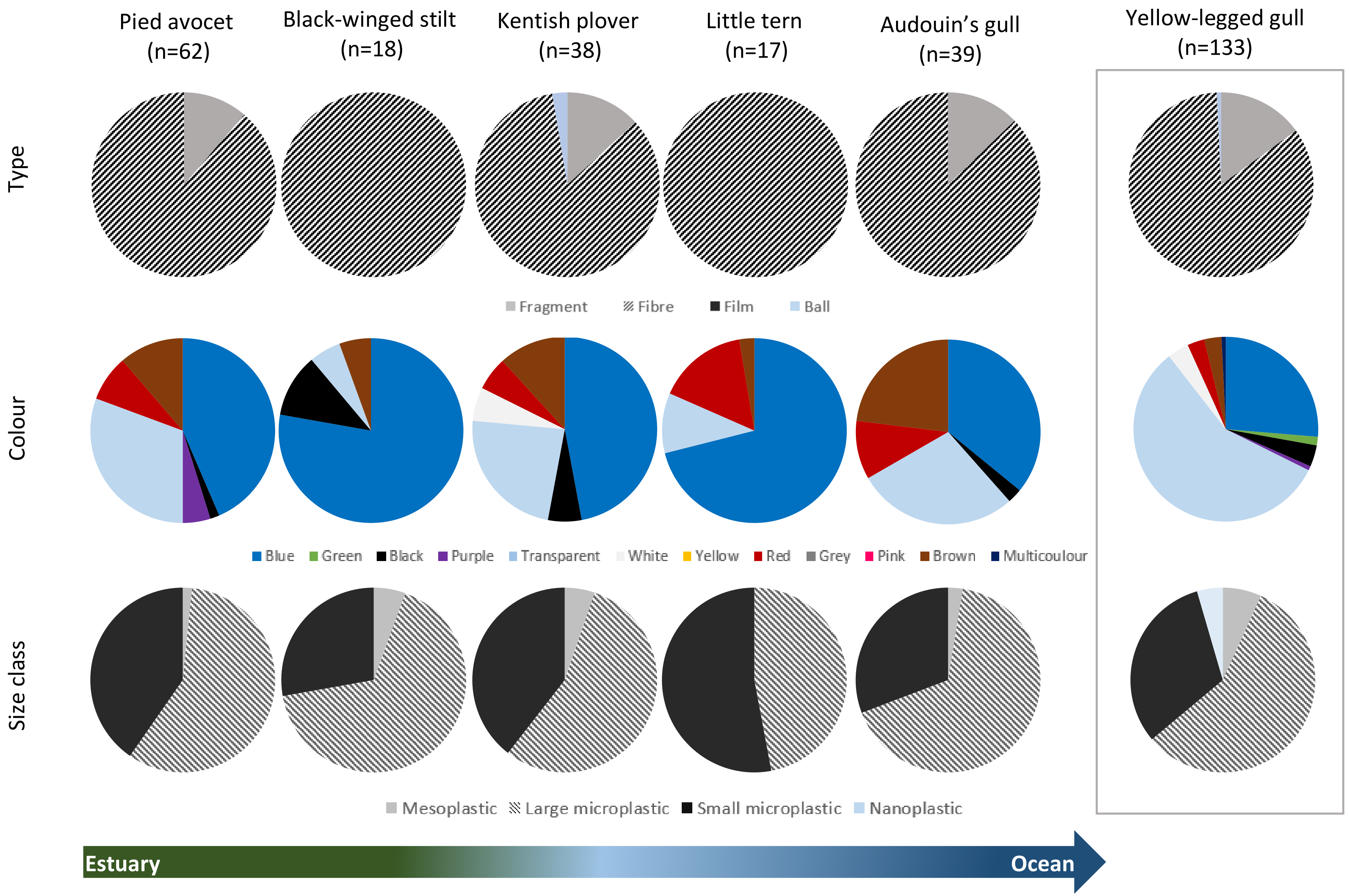

Figure 11 – Descriptive analysis of the plastics found in the faecal samples of each species. Total number of plastics found are shown at the top of the figure. First row shows the pie plots of types of plastics found in the samples; Second row describes percentage of colours found; Third row shows the percentage of each size class of the plastics found (5mm<Mesoplastic<25mm; 1mm<LargeMP<5mm; 1µm<SmallMP<1mm; 1nm<Nanoplastic<1µm). Arrow at the bottoms represents the gradient of relative ecological niches occupied by each species. Due to the Yellow-legged gulls' generalist foraging behaviour, it is presented aside, inside the grey outline.

Analysis of the NMDS axis for the colours (Fig.12b) shows no clear separation among the different species samples. Additionally, centroids of the samples are closely located to each other with a tendency to be more closely associated to the colour blue. However, there seems to be a clear separation across the NMDS axis 1, between the centroids of the yellow-legged gull and the Black-winged stilt. The Yellow-legged gull samples were more closely related to the colour "transparent" and "white" while the Black-winged stilt was more associated to the colour "blue" and "black".

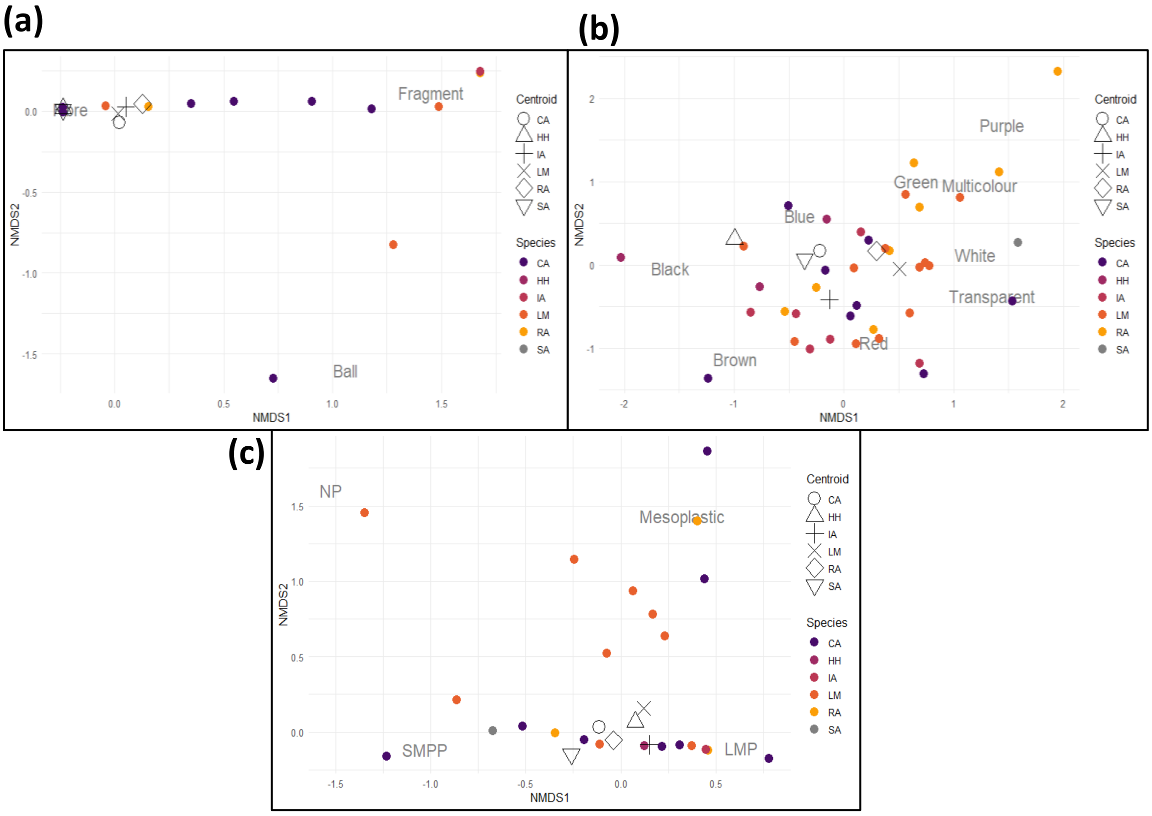

Figure 12 – Non-metric multidimensional scaling plots using faecal sample data (n=99, only positive samples considered). Each point represents a faecal sample. Some points are overlapped. (**a**) Types of plastics found; (**b**) Colours; (**c**) Size classes (5mm<Mesoplastic<25mm; 1mm<LMP<5mm; 1µm<SMP<1mm; 1nm<NP<1µm). Centroids of the species are shown in each plot. Species presented are as follows: CA – Kentish plover, HH – Black-winged stilt, IA – Audouin's gull, LM – Yellow-legged gull, RA – Pied avocet, SA – Little tern.

Table 2 – Generalized Linear models used to identify the effect of species (HH – Black-winged stilt, RA – Pied avocet, CA – Kentish plover, SA – Little tern, IA – Audouin's gull, LM – Yellow-legged gull) on the presence and absence on the overall occurrence of plastics, fibres and fragments, presence and absence of the colours blue, transparent, red, and brown in faecal samples. Binomial distribution was used. Associated ß±SE, Z and p values are presented. Highlighted in red are significant values (p<0.05). The Kentish plover (CA) is the reference species.

Binomial models (Occurrence)							
Presence overall		CA	HH	IA	LM	RA	SA
	ß		1.04	0.45	0.73	0.37	-0.51
	SE (±)		0.88	0.49	0.50	0.48	0.49
	Z		1.19	0.93	1.46	0.78	-1.04
	p		0.235	0.353	0.145	0.437	0.299
Fibre							
	ß		1.15	0.43	0.70	0.24	-0.41
	SE (±)		0.88	0.49	0.49	0.48	0.49
	Z		1.31	0.89	1.41	0.49	-0.82
	p		0.190	0.374	0.158	0.622	0.412
Fragments							
	ß			0.64	0.42	0.38	
	SE (±)			0.69	0.72	0.72	
	Z			0.92	0.59	0.54	
	p			0.355	0.556	0.590	
Blue							
	ß		0.38	-0.13	0.38	0.07	-1.04
	SE (±)		0.78	0.49	0.48	0.48	0.56
	Z		0.49	-0.25	0.78	0.16	-1.85
	p		0.624	0.796	0.432	0.873	0.064
Transparent							
	ß		0.16	1.17	2.23	1.28	-0.56
	SE (±)		1.19	0.66	0.63	0.65	0.90
	Z		0.14	1.77	3.50	1.96	-0.62
	p		0.890	0.075	<0.001	0.049	0.532
Red							
	ß		-15.45	0.16	-0.59	0.38	-0.56
	SE (±)		1398.72	0.75	0.90	0.71	0.90
	Z		-0.01	0.22	-0.66	0.54	-0.62
	p		0.991	0.827	0.507	0.590	0.532
Brown							
	ß		1.64	2.64	1.31	2.44	0.91
	SE (±)		1.47	1.08	1.18	1.09	1.25
	Z		1.12	2.43	1.11	2.24	0.73
	p		0.266	0.014	0.265	0.025	0.466

Table 3 – Generalized Linear models used to identify the effect of species (HH – Black-winged stilt, RA – Pied avocet, CA – Kentish plover, SA – Little tern, IA – Audouin's gull, LM – Yellow-legged gull) on the overall number of plastics, number of fibres and fragments, number of blue, transparent, red, and brown plastics in faecal samples. For each response variable the model used is identified at the top of each sub-table. Associated ß±SE, Z and p values are presented. Highlighted in red are significant values ($p<0.05$). Note that since the Black-winged stilt and Little tern had no fragments, no values are shown. The Kentish plover (CA) is the reference species.

		CA	HH	IA	LM	RA	SA
Total		Zero inflated with negative binomial distribution					
	ß		0.67	0.06	1.56	0.71	-0.73
	SE (±)		0.49	0.38	0.36	0.39	0.42
	Z		1.38	0.16	4.27	1.85	-1.73
	p		0.167	0.871	<0.001	0.063	0.082
Fibre		Zero inflated with negative binomial distribution					
	ß		0.95	0.2	1.68	1.04	-0.45
	SE (±)		0.44	0.32	0.31	0.36	0.36
	Z		2.15	0.64	5.31	2.93	-1.25
	p		0.031	0.521	<0.001	0.003	0.211
Fragment		Zero inflated with negative binomial distribution					
	ß			-0.41	2.51	0.47	
	SE (±)			1.34	1.26	1.41	
	Z			-0.31	1.98	0.33	
	p			0.757	0.047	0.737	
Blue		Zero inflated with negative binomial distribution					
	ß		1.20	-0.72	0.51	0.28	-1.25
	SE (±)		0.58	0.48	0.45	0.47	0.54
	Z		2.08	-1.48	1.15	0.59	-2.32
	p		0.037	0.137	0.251	0.552	0.020
Transparent		Zero inflated with negative binomial distribution					
	ß		0.14	1.16	3.50	1.96	2.27
	SE (±)		1.17	0.63	0.6	0.73	1.13
	Z		0.12	1.85	5.79	2.67	2.01
	p		0.900	0.064	<0.001	0.007	0.044
Red		Poisson					
	ß		-15.48	-0.26	-0.26	-0.29	-1.61
	SE (±)		1226.07	0.64	0.65	0.65	1.08
	Z		-0.01	-0.40	-0.40	-0.45	-1.49
	p		0.990	0.687	0.687	0.652	0.135
Brown		Poisson					
	ß		1.53	2.34	1.53	2.06	0.87
	SE (±)		1.41	1.05	1.12	1.07	1.22
	Z		1.08	2.22	1.37	1.92	0.71
	p		0.278	0.026	0.171	0.054	0.477

Sizes

The overall mean size of the plastics found in the faeces was of 1.83 ± 1.72 mm. The Black-winged stilt presented the largest plastics (2.13±1.83 mm) followed by the Yellow-legged gull (2.09±2.02 mm) (Table 1). When categorized into size classes, the overall FO was the highest for large microplastics (1mm<LMP<5mm) (41.62%, Table 4). Notably, almost all species (5 out of 6) had at least one sample with mesoplastic(s), whereas nanoplastics only occurred in one of the species (Yellow-legged gull) (Table 5, Fig.10d). Out of the 307 plastics found, 14 were mesoplastics (5mm<Mesoplastic <25mm), 179 large microplastics (1mm<LMP<5mm), 108 small microplastics (1µm<SMP<1mm) and 6 were nano plastics (1nm<NP<1µm) (Table S13-Supplementary material). The Yellow-legged gull had the highest amount of large microplastics (LMP, n=76) followed by the Pied avocet (n=36), the Audouin's gull (n=26) and the Kentish plover (n=21). By contrast, the Black-winged stilt (n=12) and the Little tern (n=8) had the lowest amount. Small microplastics (SMP) amounts were the highest in the Yellow-legged (n=42) followed by the Pied avocet (n=25). The Kentish plover (n=15), Audouin's gull (n=12), Little tern (n=9) and the Black-winged stilt (n=5) samples revealed lower amounts. The Pied avocet had the largest proportion of large microplastics (66,67%, n=36) followed by the Yellow-legged gull (57.14%, n=76) (Table 5). Furthermore, the Pied avocet had the highest proportion of small microplastics (40.32%, n=25) followed by the Kentish plover (39.47%, n=15) (Table 6, Fig. 11).

Table 4 – Generalized Linear Model on the effect of the bird species (HH – Black-winged stilt, RA – Pied avocet, CA – Kentish plover, SA – Little tern, IA – Audouin's gull, LM – Yellow-legged gull) on the size of the plastics found in faecal samples. GLM with gaussian fitting and identity distribution. ß±SE, Z and p values associated to each species are presented. Highlighted in red are significant values (p<0.05). The Kentish plover (CA) is the reference species.

	Faeces					
	CA	HH	IA	LM	RA	SA
Size (mm)						
ß		0.39	0.03	0.35	-0.29	-0.46
SE (±)		0.49	0.39	0.31	0.35	0.49
Z		0.80	0.08	1.12	-0.85	-0.92
p		0.422	0.937	0.264	0.396	0.360

GLMs with a Gaussian fitting using the sizes of each plastic revealed no statistical differences when compared to the reference species (Table 4). Furthermore, NMDS analyses based on size classes of plastics in the faeces have shown no real segregation between species. All centroids were closely positioned (Fig. 12c). Notably, the Yellow-legged gull 's centroid was positioned higher in the NMDS2 axis mainly due to some samples having mesoplastic sized contents (Fig. 12c).

Correlations between: (1) size of plastics (mm) in faecal samples and mean weight (g) of each species (rs=0.42, p = 0.39); (2) the number of plastics per species faecal samples and the mean weight (g) of each species (rs=0.77, p = 0.07), were, both, not significant (Table S13, S14 – Supplementary material). However, the correlation between the weight (g) of the faecal samples and the number of plastics found in them was significant (rs=0.28, p=0.04) (Table S15) (Fig. 13a). This, however, could be explained by the fact that this was not a six-point correlation like those presented before. Notably, the correlation between the median weight of the birds and the total number of plastics found in the faeces appears to close to significant (p=0.07 – Table S14 – Supplementary material). A scatterplot of the relation was, thus, also presented (Fig. 13b).

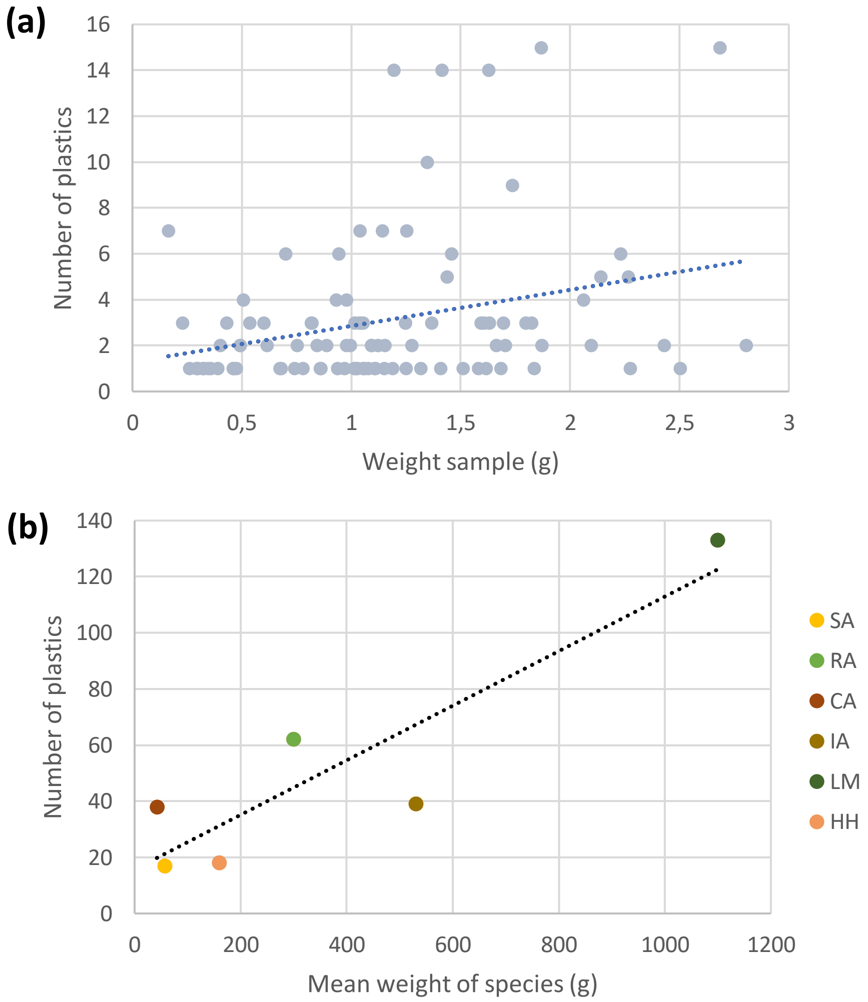

Figure 13 - (**a**) Scatterplot presenting the relationship between the number of plastics found in faeces and the weight of each faecal sample (n=99). Only positive samples were considered. Spearman's rank correlation coefficients, rs=0.28, p=0.004; (**b**) Scatterplot between the mean number of microplastics found per species (HH – Black-winged stilt, RA – Pied avocet, CA – Kentish plover, SA – Little tern, IA – Audouin's gull, LM – Yellow-legged gull) and the mean weight of each species. Near significant correlation (Spearman's rank correlation coefficients rs=0.07, p=0.07).

Table 5 – Frequency of occurrence (%) of each category of plastics across bird species (HH – Black-winged stilt, RA – Pied avocet, CA – Kentish plover, SA – Little tern, IA – Audouin's gull, LM – Yellow-legged gull) in both types of samples (faeces and regurgitates). Relative ecological niche is shown above each species name. Number of samples where at least one plastic was present (n) is presented next to the percentage of occurrence. Total number of samples analyzed (n) for each species is shown at the bottom. Overall FO of each category shown on the right side of each sub-table.

	Frequency of Occurance (%)										
	Faeces							Regurgitates			
	Estuarine		Transition		Ocean	Mixed		Transition	Ocean	Mixed	
	HH	RA	CA	SA	IA	LM	Overall	SA	IA	LM	Overall
Occurance (%)	75.00 (n=6)	60.60 (n=20)	51.35 (n=19)	38.75 (n=12)	62.50 (n=20)	68.75 (n=22)	57.20 (n=99)	43.83 (n=32)	3.38 (n=2)	18.18 (n=14)	22.96 (n=48)
Type											
Fragment	-	15.15	10.81	-	18.75	15.63	11.56	9.59	-	9.09	6.70
Fibre	75.00	54.55	48.65	38.71	59.37	65.63	54.34	39.73	1.69	2.60	15.31
Filament	-	-	-	-	-	-	-	-	-	-	-
Sfere	-	-	-	-	-	-	-	-	-	-	-
Film	-	-	-	-	-	-	-	-	1.69	9.09	3.83
Ball	-	-	2.70	-	-	3.13	1.16	1.37	-	3.90	1.91
Colour											
Blue	50.00	42.42	40.54	19.35	37.50	50.00	38.73	31.51	1.69	3.90	12.92
Green	-	-	-	-	-	3.13	0.58	1.37	-	9.09	3.83
Black	25.00	3.03	-	3.23	3.13	12.50	5.20	-	-	2.60	0.96
Purple	-	9.09	-	-	-	3.13	2.31	2.74	-	-	0.96
Transparent	12.50	30.30	10.81	6.45	28.13	53.13	24.86	4.11	1.69	5.19	3.83
White	-	-	-	3.23	-	9.38	2.31	1.37	1.69	5.19	2.87
Yellow	-	-	-	-	-	-	-	-	-	-	-
Red	-	18.18	10.81	6.45	12.50	6.25	10.40	4.11	-	2.60	2.39
Grey	-	-	-	-	-	-	-	-	-	-	-
Pink	-	-	-	-	-	-	-	-	-	-	-
Brown	12.50	24.24	2.70	6.45	28.13	9.38	13.87	10.96	-	3.90	5.26
Multicoulour	-	-	-	-	-	3.13	0.58	-	-	-	-
Size											
Mesoplastic	12.50	3.03	5.41	-	3.13	15.63	5.78	1.37	3.39	18.18	8.13
LargeMP	62.50	39.39	32.43	22.58	50.00	59.38	41.62	35.62	-	3.90	13.88
SmallMP	50.00	39.39	32.43	22.58	28.13	40.63	33.53	21.92	-	-	7.60
NanoPlastic	-	-	-	-	-	6.25	1.16	-	-	-	-
n	8	33	37	31	32	32	173	73	77	59	209

Table 6 – Percentage of (micro)plastics (%) from each category detected in the bird species samples (HH – Black-winged stilt, RA – Pied avocet, CA – Kentish plover, SA – Little tern, IA – Audouin’s gull, LM – Yellow-legged gull). Relative ecological niche is shown above each species name. Number of plastics found in the species corresponding are shown at the top (n - plastics).

		Percentage microplastics found (%)								
		Faeces						Regurgitates		
		Estuarine		Transition		Ocean	Mixed	Transition	Ocean	Mixed
		HH	RA	CA	SA	IA	LM	SA	IA	LM
n (plastics)		18	62	38	17	39	133	276	4	56
Type										
	Fragment	-	11.29	13.16	-	12.82	15.04	82.97	-	46.43
	Fibre	100.00	88.71	84.21	100.00	87.18	84.21	17.03	25.00	8.93
	Filament	-	-	-	-	-	-	-	-	-
	Sfere	-	-	-	-	-	-	-	-	-
	Film	-	-	-	-	-	-	-	75.00	39.29
	Ball	-	-	2.63	-	-	0.75	-	-	5.36
Colour										
	Blue	77.78	43.55	71.05	47.06	35.90	26.32	92.39	25.00	5.36
	Green	-	-	-	-	-	1.50	0.36	-	30.36
	Black	11.11	1.61	-	5.88	2.56	3.76	-	-	8.93
	Purple	-	6.45	-	-	-	0.75	0.72	-	-
	Transparent	5.56	30.65	1.53	23.53	28.21	57.14	1.40	50.00	25.00
	White	-	-	-	5.88	-	3.76	0.36	25.00	8.93
	Yellow	-	-	-	-	-	-	-	-	-
	Red	-	6.45	15.79	5.88	10.26	3.01	1.09	-	14.29
	Grey	-	-	-	-	-	-	-	-	-
	Pink	-	-	-	-	-	-	-	-	-
	Brown	5.56	11.29	2.63	11.76	23.08	3.01	3.62	-	7.14
	Multicoulour	-	-	-	-	-	0.75	-	-	-
Size										
	Mesoplastic	5.56	1.61	5.26	-	2.56	6.77	-	100.00	78.57
	LargeMP	66.67	58.06	55.26	47.06	66.67	57.14	13.41	-	21.43
	SmallMP	27.78	40.32	39.47	52.94	30.77	31.58	86.23	-	-
	NanoPlastic	-	-	-	-	-	4.50	-	-	-

Table 7 - Plastics detected in regurgitate samples of waterbirds in Ria Formosa, Portugal. Total number of regurgitates analysed, number of plastics detected, plastics' frequency of occurrence (%), mean number of plastics (±SD) in the affected samples, mean size (±SD) and overall mean number (±SD) (all samples) per species.

Species	Total n (samples)	Occurence	Total n (micro)plastics	Mean number of plastics per affected sample (±SD)	Mean size (mm) (±SD)	Total mean ammount (±SD)
Little tern (SA)	73	43.84	276	8.6 ± 28.93	0.82 ± 1.52	3.70 ± 19.62
Yellow-Legged gull (LM)	77	18.18	56	4 ± 4.67	15.69 ± 16.10	0.70 ± 2.52
Audouin's Gull (IA)	59	3.39	4	2 ± 1	13.50 ± 5.80	0.07 ± 0.41
Overall	209	22.90	336	7 ± 23.87	3.46 ± 8.82	1.60 ± 11.81

3.3 - Regurgitates

In total 336 plastics were found across 209 regurgitate samples. The Little tern (SA) samples had 276 microplastics (n=73 samples), the Yellow-legged gull (LM) had 56 (n=77 samples) and the Audouin's gull (IA) had 4 (n=59 samples).The Little tern had the largest mean value of plastics per positive sample (8.60 ± 28.93), followed by the Yellow-legged gull (4 ± 4.67) and the Audouin's gull (2 ± 1) (Table 7). Please note that the high SD in the Little tern regurgitates is largely due to one of the samples having 166 plastics (Table S13- Supplementary material). The Yellow-legged gull had the largest mean size (15.69 ± 16.10 mm) followed by the Audouin's gull (13.50 ± 5.80 mm) and finally by the Little tern (0.82 ± 1.52 mm) (Table 7).

Overall, FO of plastics among the species was of 22.96%. The FO in the Little tern was 43.84%. The Yellow-legged gull regurgitates showed a FO of 18.18% and the Audouin's gull, 3.39% (Fig. 14a). Binomial GLMs, using the presence and absence of plastics in the samples among the species regurgitates revealed that the Audouin's gull had significantly less prevalence of plastics in their samples, whereas the Little tern samples the presence was significantly higher (Table 8). Furthermore, GLMs using the total number of (micro)plastics found in the regurgitates in each species, revealed a near significant difference of amounts in the Little tern. For the remaining species no significant differences were found (Table 9).

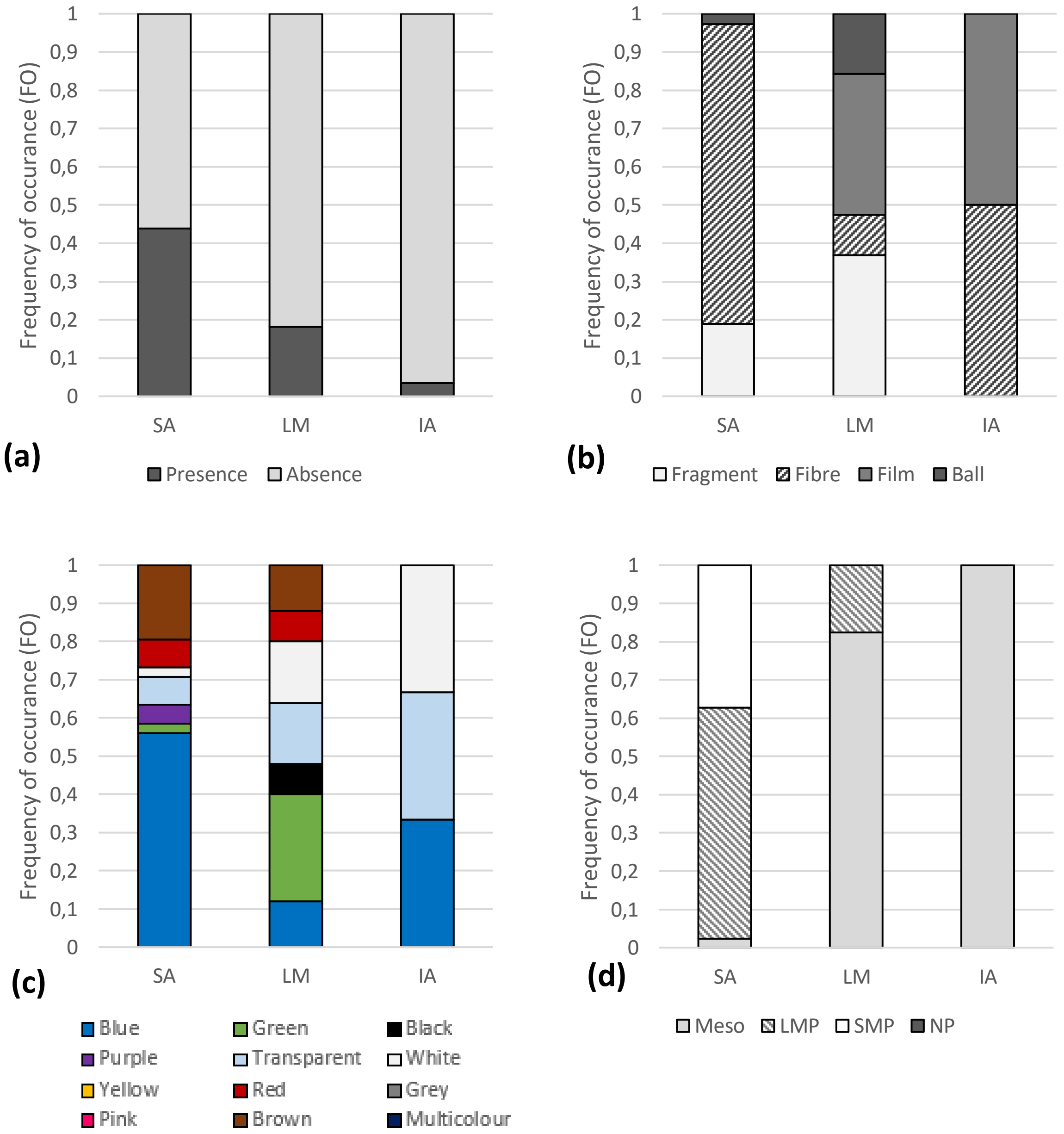

Figure 14 – Bar plots showing the Frequency of occurrence (FO) of each category of plastic found in across the species (SA – Little tern, LM – Yellow-legged gull, IA – Audouin's gull). Values adjusted to 1 (100%) Formulas used can be found in the statistical methods. (**a**) FO of samples with at least one plastic across the 6 species (FO); (**b**) Frequency of occurrence of the types of plastics; (**c**) Frequency of occurrence of the different plastic colours; (**d**) Frequency of occurrence of plastic size classes (5mm<Meso<25mm; 1mm<LMP<5mm; 1µm<SMP<1mm; 1nm<NP<1µm).

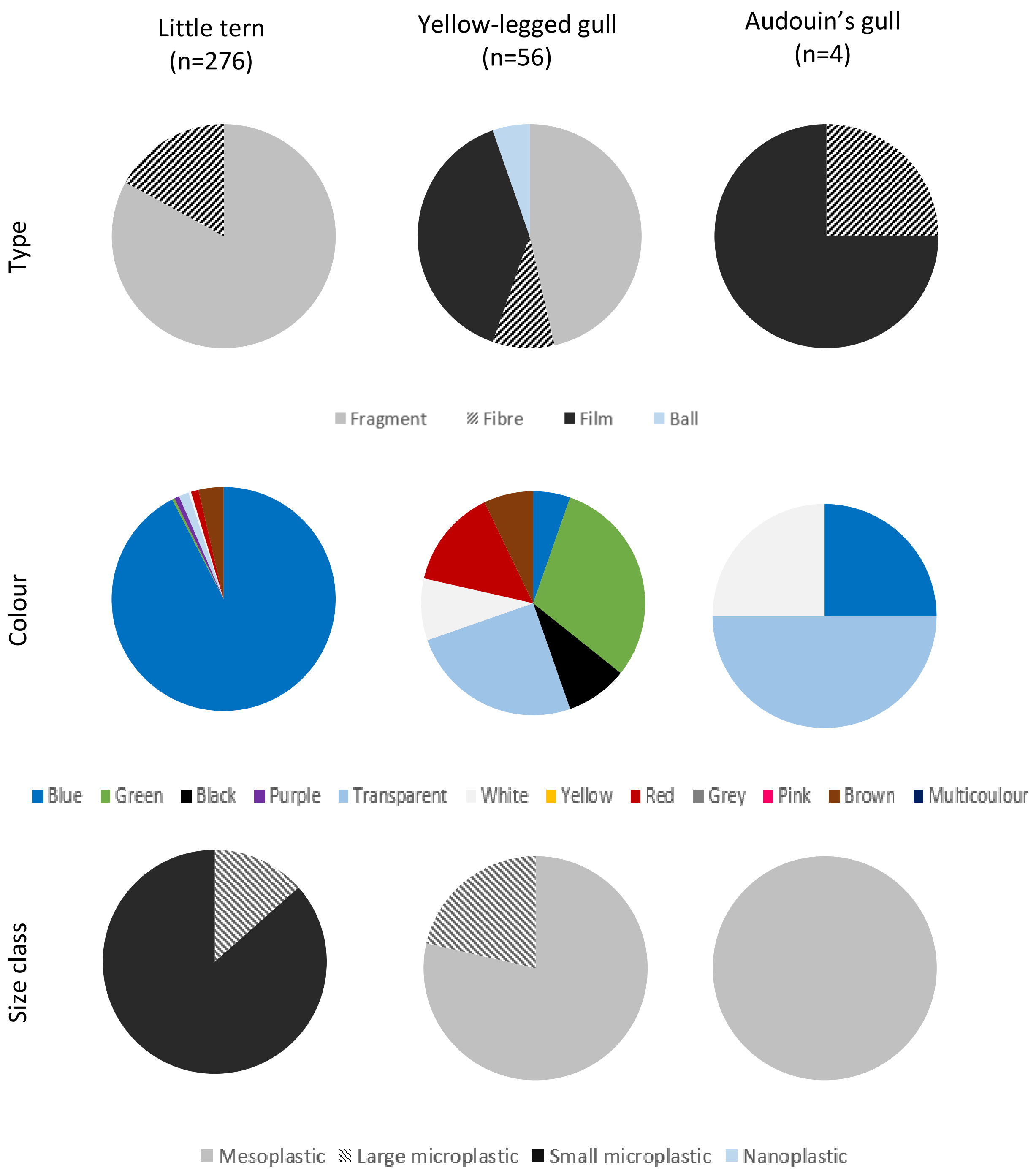

Figure 15 – Descriptive analysis of the plastics found in the regurgitates. Total number of plastics found shown at the top of the image. First row shows the pie plots of types of plastics found in the samples; Second row describes percentage of colours found; Third row shows the percentage of each size class of plastics found (5mm<Mesoplastic<25mm; 1mm<largeMP<5mm; 1µm<SmallMP<1mm; 1nm<Nanoplastic<1µm).

3.3.1 - Plastic characteristics in regurgitates

Type

Similar to the faecal samples, the most common type of plastics in the regurgitates were fibres, being present in all 3 species. The overall FO of fibres was of 15.31% (Table 5). Frequency of occurrence ranged from 39.73% in the Little tern (n=32) to 1.69% in the Audouin's gull (n=2) (Table 5). Fragments were also common, with a FO ranging from 9.59% in the Little tern to 9.09% in the (Yellow-legged gull). No fragments were found in Audouin's gull regurgitates (Table 5, Fig. 14b). The Yellow-legged gull had the most diverse repertoire of plastic types in the regurgitates (4 out of 6). No filaments nor spheres were found. GLMs using a binomial fitting performed with the presence/absence of fibres in each sample revealed that the Little tern had a significantly higher ($p<0.05$) presence when compared to the reference species (Table 8).

Fragments proportions were the highest type found across the species, representing 82.97% (n=229) of the plastics in the Little tern and 46.43% (n=26) in the Yellow-legged gull (Table 6, Fig. 15). Fibres represented 25% (n=1) of the plastics found in the Audouin's gull, 17.03% (n=47) in the Little tern, and 8.93% (n=5) in the Yellow-legged gull. Notably, films were present in both gull species representing 39.29% (n=22) in the Yellow-legged gull and 75% (n=3) in the Audouin's gull (Table 6, Fig. 15, Table S13 – Supplementary material). GLMs using zero inflated fitting showed that the Little tern and Yellow-legged gull (by this order) had significantly higher amounts of fibres ($p<0.05$) (Table 9).

Results from the NMDS (Fig.16a) displayed a clear separation across the species centroids. The Little tern (SA) centroid was more closely associated with fibres, while the Audouin's gull (IA) and Yellow-legged gulls' (LM) centroid were associated with fragments and films

Colour

The colour blue was the most common and prevalent among the species with an overall FO of 12.92%. All other colours had an occurrence below 10%. The Little tern had a FO of 31.51% for the colour "blue", followed by 10.96% of the colour "Brown" (Table 4, Fig. 14c). The Yellow-legged gull had the highest occurrence of the colour green (9.09%) followed by transparent and white (both accounting for 5.19%) (Table 4, Fig. 14c). Furthermore, the Yellow-legged gull also had the most diverse repertoire of colours (7 out of 12). Binomial GLMs for the colour “blue” showed that the Little tern had a significantly higher presence of blue coloured (micro)plastics in their regurgitates when compared to the reference species (Table 8).

Plastics found in the Little tern regurgitates were mostly blue (92.39%, n=255). In the Audouin’s gull, 50% (n=2) were transparent, 25% (n=1) were blue and the remaining 25% (n=1) were white. Most plastics found in the Yellow-legged gull were green (30%, n=17) followed by the colour transparent (25%, n=14) and lastly, the colour red (14.29%, n=8) (Table 5, Fig. 15). The remaining percentages were distributed between brown (n=4), white (n=5), black (n=5) and blue (n=3). The Yellow-legged gull and the Little tern showed the broadest repertoire of colours in their regurgitates (7 out of 12 colours for both species) (Table S13 – Supplementary material).

Table 8 – Generalized Linear models used to identify the effect of species (SA – Little tern, IA – Audouin’s gull, LM – Yellow-legged gull) on the presence and absence on the overall occurrence of plastics, fibres, presence and absence of the colours blue in the samples. Binomial distribution was used. Associated ß±SE, Z and p values are presented. Highlighted in red are significant values ($p<0.05$). The Audouin’s gull (IA) is the reference species.

		Binomial models (Occurrence)		
Presence overall		IA	LM	SA
	ß		1.84	3.10
	SE (±)		0.78	0.76
	Z		2.37	4.09
	p		0.017	<0.001
Fibres				
	ß		0.44	3.64
	SE (±)		1.24	1.04
	Z		0.35	3.51
	p		0.724	<0.001
Blue				
	ß		0.85	3.28
	SE (±)		1.17	1.04
	Z		0.73	3.16
	p		0.464	<0.001

Table 9– Generalized Linear models used to evaluate the effect of bird species (IA – Audouin's gull, LM – Yellow-legged gull, SA – Little tern) on the presence and quantity of plastics and their types and colours (Fibre, Blue) in regurgitates. Models used are identified at the top of each sub-table. Associated ß±SE, Z and p values are presented. Highlighted in red are significant values (<0.05). The Audouin's gull (IA) is the reference species.

		IA	LM	SA
Total		Negative bi. Distribution		
	ß		1.25	2.47
	SE (±)		1.39	1.35
	Z		0.90	1.83
	p		0.366	0.067
Fibre		Zero inflated		
	ß		4.18	4.12
	SE (±)		1.72	1.60
	Z		2.43	2.57
	p		0.015	0.010
Blue		Negative bi. Distribution		
	ß		1.93	5.33
	SE (±)		1.19	1.15
	Z		1.62	4.64
	p		0.105	<0.001

GLMs using zero inflated model with negative binomial distribution (Table 9) using the number of blue coloured plastics per sample and species revealed that the Little tern had significantly higher amounts of blue coloured (micro)plastics.

Results from the NMDS (Fig. 16b) showed a clear separation along the first axis (NMDS1) between the colour blue and the other colours found in the samples (Green, red, white, transparent, and brown). In the second axis (NMDS2) a clear segregation is noticeable between the colour brown and the rest of the colours. Additionally, the colour blue also seems to be separated from the rest of the colours through the NMDS2 axis. Centroids show that the Yellow-legged gull was more closely associated with the colours white and transparent, whereas the Audouin's gull and the Little tern were more associated with the colour blue.

Size

Large microplastics were the most common (FO=13.88%) followed by mesoplastics (FO=8.13%) and small microplastics (FO=7.60%). The Yellow-legged gull showed the highest FO of mesoplastics (18.18%) and the Little tern had the highest FO of large microplastics (35.62%) and small microplastics (21.92%) (Table 5, Fig. 14d). Plastics present in the Yellow-legged gull regurgitates were mainly mesoplastics (78.57%, n=44) (Table 6, Table S13 – Supplementary material). In Audouin's gull regurgitates all plastics found were mesoplastics (100%, n=4). The Little tern had predominantly smaller microplastics (86.13%, n=238). The remaining percentage included large microplastics (13.41%, n=37) (Table 6, Fig. 15).

Table 10– Effect of variable species (IA – Audouin's gull, LM – Yellow-legged gull, SA – Little tern in the size of the microplastics found in regurgitates samples and across the species. GLM with gaussian fitting and identity distribution. ß±SE, Z and p values associated to each species are presented. Highlighted in red are significant values ($p<0.05$). The Audouin's gull (IA) is the reference species.

	Regurgitates		
	IA	LM	SA
Size (mm)			
ß		2.19	-12.67
SE (±)		3.53	3.43
Z		0.62	-3.69
p		0.534	<0.001

The GLM performed using a Gaussian fit and identity distribution (Table 10) showed significant differences exclusively for the Little tern (SA). Little tern plastics were significantly smaller whereas the Audouin's gulls' plastics were significantly larger.

Finally, the NMDS representation (Fig. 16c) of the size classes revealed clear separations in both axes between all three bird species. The Little tern (SA) was more closely associated with large microplastics, despite having some samples being close to small microplastics. Both gull species showed a close relation to the mesoplastic size class (centroids were closely positioned).

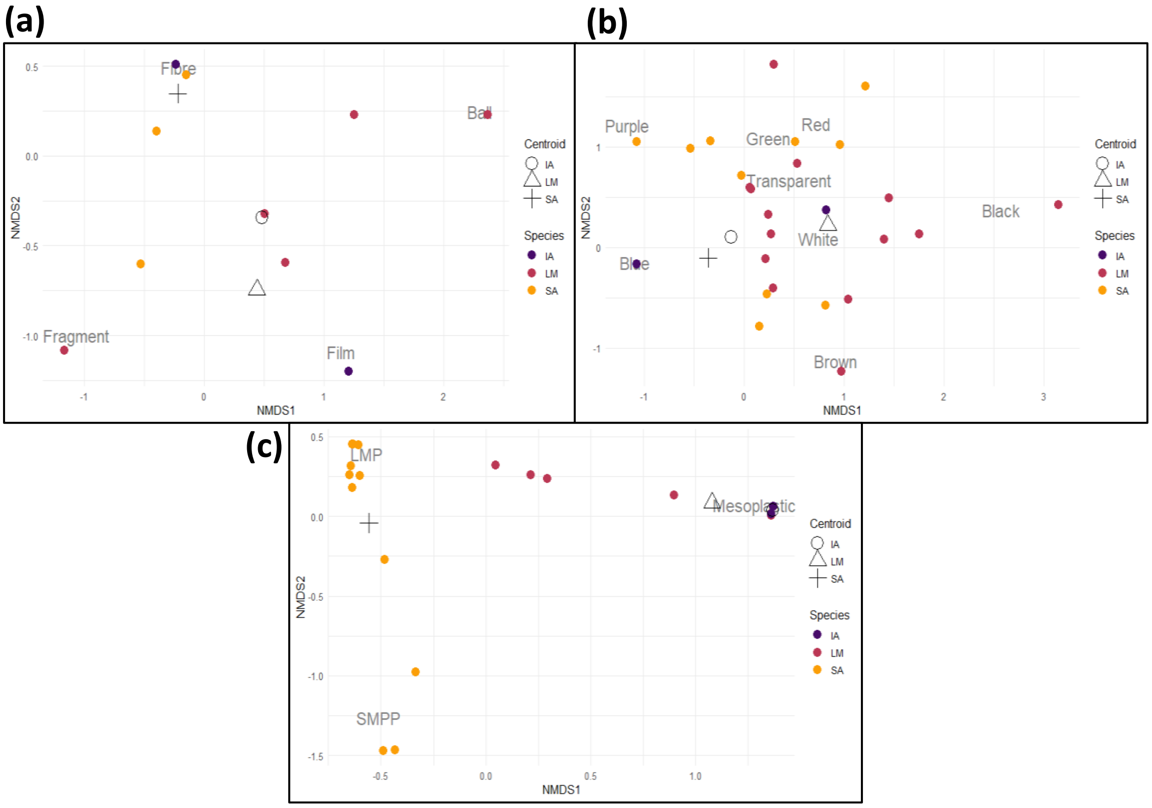

Figure 16 – Non-metric multidimensional scaling using regurgitate data (n=48) for (**a**) Types of plastics, their (**b**) colours and (**c**) size classes (5mm<Mesoplastic<25mm; 1mm<LMP<5mm; 1µm<SMP<1mm; 1nm<NP<1µm). Each dot represents an regurgitate sample. Some dots are overlapped. Centroids are shown for each of the species. Species presented are as follows: IA – Audouin's gull, LM – Yellow-legged gull, SA – Little tern.

3.3 - Additives

Proportions of the mean concentrations, and cumulative concentrations (ng/500mg of egg sample) of each of the PBDE's found in the eggs of each species are shown in Fig. 17. The dominant type of PBDE found was BDE 28, ranging from 91.37% in the Pied Avocet to 80.39% in the Black winged stilt. The second most common was BDE 47 ranging from 9.65% (Black-winged stilt - HH) to 3.40% (Pied avocet). Cumulative concentrations revealed to be the highest in the Black-winged stilt, Little tern and the Yellow-legged gull.

Axis 1 of the PCA used for the analysis of the additives in the eggs of the different bird species explained 45.1% of the variance and axis 2 explained 23.0% (Fig. S7 - Supplement). Samples from the Black-winged stilt appear to be more closely associated with BDE 154, 47 and 99 and explained most of the variability of these 3 compounds (Fig. 18). This suggests larger amounts of these chemicals in this species. The Little tern (SA) also seems to be more closely associated to BDE 28 when compared to the Black-winged stilt, implying that the Little tern eggs likely had larger amounts of BDE28. Audouin's gull samples look more closely positioned to the BDE 28,153 and 183, also suggesting a larger uptake of these compounds (Fig. 18). The Kentish plover samples appear to be dispersed, probably explained by a high variability in PBDEs concentrations. Some samples were closely associated with BDE 28 (higher concentration of BDE 28) while others were more closely positioned near the BDE 47 and 99 (higher concentration of BDE 47 and 99). Lastly, the Pied avocet seems to be more closely associated to BDE 28 and some samples to BDE 153 and 183, having, however, some samples with lower concentrations of these compounds. The only noticeable segregation between species seems to be across Axis 2 where it separates a large portion of Black-winged stilt samples from the major conglomerate of samples (Fig. 18).

Six-point Spearman correlation analysis between the mean scores on the first axis (PC1) of each bird species and the total number of plastics detected in their faeces was not significant (r=0.60, p=0.20,), suggesting that there is no relationship between higher numbers of plastics consumed and additives assimilated (Table S16 – Supplementary material).

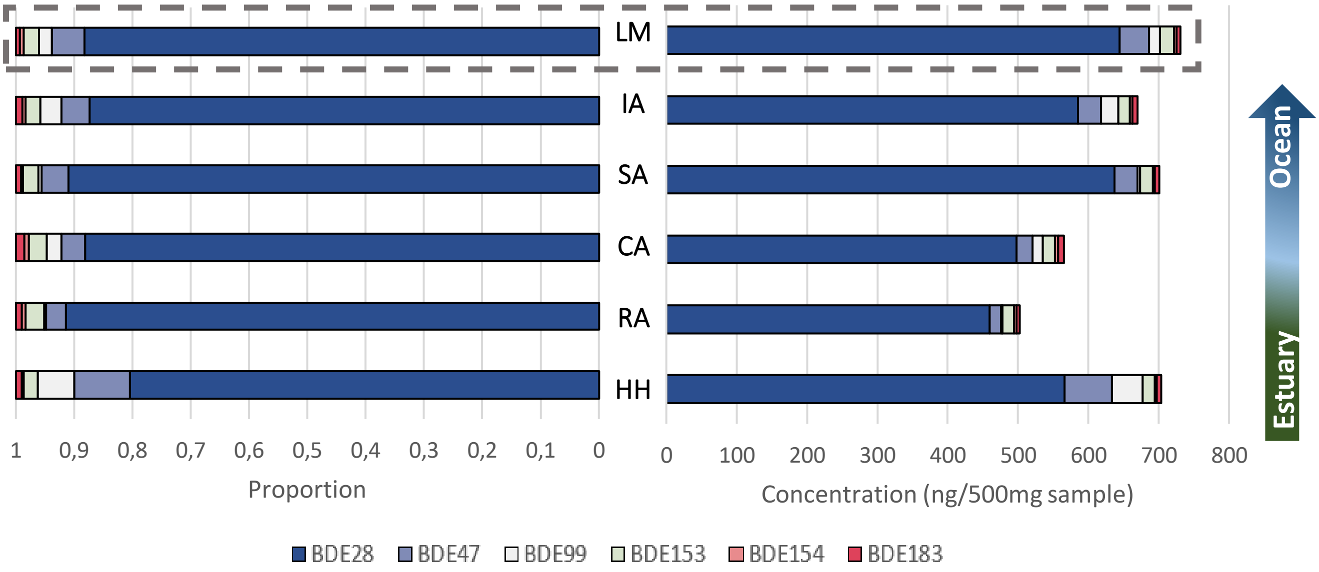

Figure 17 – Bar plot with the corresponding proportions of each PBDE found in the eggs of each species sampled in Ria Formosa, Portugal (left) and corresponding cumulative concentrations (ng/500mg sample - right). Species presented are listed as follows: LM – Yellow-legged gull; IA – Audouin's gull; SA – Little tern; CA – Kentish plover; RA – Pied avocet; HH – Black-winged stilt. Arrow on the right represents the establish gradient of species based on their relative ecological niches. Due to the Yellow-legged gull's generalist habits a gray box is represented around it.

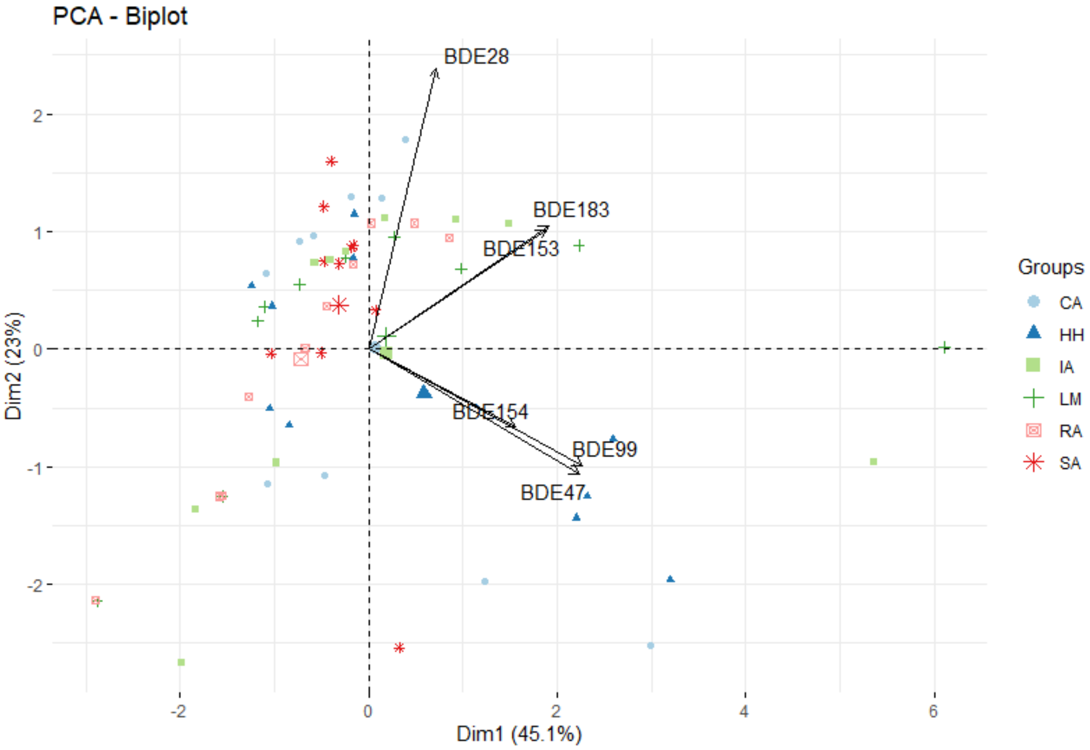

Figure 18 - Principal component analysis, representing the bird species in different shapes and the additives present in the bird's eggs as vectors. Each point represents an egg sample (n=10 for each species, total n=60). Axis 1 explains 45.1% of the variance while Axis 2 explains 23%. Species presented are listed as follows: CA – Kentish plover; HH – Black-winged stilt; IA – Audouin's gull; LM – Yellow-legged gull; RA – Pied avocet; SA – Little tern.

Chapter IV – Discussion

4.1 - Discussion

This thesis presents a first descriptive analyses of (micro)plastics and additives found in waterbird species sampled in southern Portugal. Studies such as the present are crucial for the creation of a baseline for future comparisons ultimately leading to the establishment of spatial and temporal trends of these contaminants. Mainly, it is also the first study trying to establish a gradient of plastic and additive contamination along an estuarine-sea gradient.

Such survey studies do not come without their biases When analysing data obtained from (micro)plastic contamination studies in waterbirds, it is important to note that the type of samples used (faeces, regurgitates, cadavers, stomach washes etc.) could influence the total amount of (micro)plastics found (Provencher et al., 2019). Most, if not all, sample types (including regurgitates and faeces – the main sample types used in this study) allow only for a limited view of a certain timeframe of consumption (Provencher et al.,2019). Thus, samples that had no plastics could be due to the individuals simply not consuming any (micro)plastics during the consumption period reflected in the collected sample. This does not exclude the possibility of the individual having consumed plastics before or after the timeframe reflected by the sample. The duration of the timeframe offered by these types of samples is generally influenced by species-specific, and even individual-specific factors, which are largely still not known and may influence the retention time of the contaminants. Retention periods for the sampled species are not known. Additionally, faeces and regurgitate sample types do not account for plastics that might adhere to the stomach wall of the individuals and therefore be retained for longer periods of time (Ribeiro et al., 2019). Although these sample types might not be the most suitable for an individual analysis, with a large enough sample number, an overall view of contamination of a population/species can be obtained. Thus, following the numbers proposed by Bessa et al., (2019), around 30 samples were analysed for each species as to avoid an individual bias in the overall view of the population.

Some sampling methods might also be biased when considering species that regurgitate. To avoid this, regurgitation was considered alongside the faecal samples. It is hard to infer which sample type better represents the amounts the individuals are exposed to, mainly due to the limited timeframe of consumption, mirrored in both types of samples. Therefore, the use of both sampling methods is recommended, to complement each other. Using both sample types in species that regurgitate (Yellow-legged gull, Audouin's gull, Little tern) enabled us to obtain a more complete view of consumption. This eventually uncovered an often-overlooked type of sample (regurgitates) in (micro)plastic contamination studies that revealed large amounts of consumed microplastics as evidenced in this study for the Little tern (discussed further). Furthermore, a comparison between the faeces and regurgitates could shed light into the ability of each species to regurgitate refuse. As evidenced in the present work, species that had a lower quantity of microplastics in their faeces revealed high numbers in the regurgitates and vice-versa (see below).

Besides (micro)plastic quantities, categorization of the colours might also be subject to biases. Registering the colours of the plastics can indicate probable contamination sources, thus identifying their original colours proves to be important. In this type of analysis observer classification errors leading to biases cannot be disregarded. During the cataloguing process it is likely that some colours might have been mistaken for another similar colour. Environmental biases could also have altered the original colour of the (micro)plastic, through photodegradation processes, before being ingested. Likewise, the digestive fluids of the birds can also have a role, because the acidity of the fluids can alter the colours of the plastics after ingestion. Similarly, it is plausible that, due to the digestive processes used in the laboratory to digest organic matter in the faecal samples, the colour of the (micro)plastics present could have also been altered. In the regurgitates, however, because no digestive compounds were used to process them, the risk of the colours being altered was minimised.

In general, a decreasing gradient in the amount of (micro)plastics in the species faecal samples was expected. As expected, the Yellow-legged gull had the largest amount of plastics, likely due to landfill foraging and their opportunistic habits. This species was expected to be followed by the Black-winged stilt, Pied avocet, Kentish plover, Little tern and lastly the, Audouin's gull. Results in the present study showed that the mean number of plastics in faecal samples followed a slight gradient: (ordered according to the gradient) 6.04 (Yellow-legged gull) > 3.10 (Black-winged stilt) > 3 (Pied avocet) > 2 (Kentish plover)> 1.41 (Little tern) > 2 (Audouin's gull). Yellow-legged gull faecal samples were the only samples that showed a statistically larger amount of overall plastics when compared to the other species. Notably, contrary to our initial expectations, the Little tern had less plastics than the Audouin's gull. Similar results were reflected in the presence of (micro)plastics (FO) in the faeces. A downward trend was noticeable from the estuary towards the ocean: (ordered according to the gradient) 68,75% (Yellow-legged gull) > 75% (Black-winged stilt)> 60% (Pied avocet) > 51.35% (Kentish plover) > 38.71% (Little tern) > 51.35% (Audouin's gull). Unexpectedly, the Yellow-legged gull had a lower occurrence than the Black-winged stilt. Moreover, the Audouin's gull FO was as high as the Kentish plover and higher than the Little tern. No significant differences in the presence of plastics among species were noted. Regurgitate samples showed a statistically significant decrease in both presence and in the number of plastics found across the species, with the Little tern having the highest amounts (FO = 43.84%, mean=8.6), followed by the Yellow-legged gull (FO = 18.18%, mean=4) and lastly the Audouin's gull (FO = 3.39%, mean=2).This, however, was not expected as the Yellow-legged gull was expected to have the highest FO, and largest amounts in the regurgitates out of the remaining species. It is possible that the regurgitate efficiency of the Yellow-legged gull might have influenced these results (discussed further).

Contrary to expectation, no correlation was found between the mean weight of the birds and the number of (micro)plastics in their faecal samples, as it was expected that bigger species would consume larger amounts of (micro)plastic items. However, it is possible that there could be a relationship here that we were unable to detect, as the correlation associated spearman p value was close to significance, and the R^2 value of the tendency line was close to 1. It is likely that the small number of samples analysed for the Black-winged stilt influenced this statistical result. Further studies with more species and increased sample sizes would likely reveal a significant correlation. Furthermore, no correlation was also found for bigger (heavier) species having larger plastics, indicating that the size of the plastics found is independent of the body mass of each species. Contrastingly, heavier faecal samples contained significantly more plastics. Sample weights are variable inter and intra-species indicating that heavier birds do not necessarily translate into heavier faeces. Faeces weight might be influenced by retention time and each species circadian cycles. Thus, more plastics could be present in heavier faeces independently of the species' body mass.

Besides the biases, the aforementioned results are influenced by a panoply of factors; thus, these are further discussed for each individual species in order to ascertain which environmental or species-specific factors might be contributing to the presented results.

Yellow legged gull. This species exhibited a significantly higher number of overall plastics in their faecal samples, and relatively high numbers in the regurgitates. Yellow-legged gulls have a more generalist diet than the other species, consuming prey items ranging from fish, seafood and other birds to small mammals, and anthropogenic materials (Matos et al., 2018). Gull species that have a more generalist diet and behaviour are known to experience a higher exposure and contamination by plastics (Caldwell et al., 2020). Their generalist behaviour can even be evidenced in the wide variety of plastic colours found in both types of samples analysed here.

Yellow-legged gulls are known to take advantage of fishing boats to feed on prey discarded during fishing operations. Coincidently, their foraging routes and areas are generally overlapped with those of the fishing boats. This also includes being exposed to highly polluted environments such as fishing harbours (Matos et al.,2018), where regular dumps to clean the fishing boats are performed, releasing into their vicinities unknown amounts of plastics originating from fishing gear (Wright et al., 2021; Napper et al., 2022). Yellow-legged gulls are also known to frequent waste-water treatment areas. These are areas which are known accumulation zones of microplastics (Blair et al., 2017). In fishing harbours or in wastewater treatment areas, plastics ultimately reach this species due to direct exposure through foraging in and around these areas (dermal exposure or direct consumption of contaminated water from the environment). Thus, exposure to (micro)plastics from fishing activities such as fibres originating from fishing gear (Gilman et al., 2021), fragments mostly originating from the paint of the boats (Turner et al.,2021), and numerous other particles originating from waste-water treatment plants, are likely sources of contamination for this species (Blair et al., 2017). Accordingly, significantly larger amounts of fibres (in faeces and regurgitates) and fragments (in faeces) were found when compared to the remaining species.

The largest amounts of blue coloured plastics were also found in Yellow-legged gull samples (note that statistically, no significantly higher amounts nor presence were noted), a colour generally associated with fishing activities (Gilman et al., 2021). Coincidently, it is also one of the less common colours found in ocean and pelagic environments (Hidalgo-Ruz et al., 2021), which further supports that the direct interaction of the birds with fishing operations are a source of contamination. Yet, the idea that they might have directly consumed contaminated prey or water or confounded bigger plastic particles as possible prey or food items (fish for example) should not be discarded (Blight et al., 1997; Lavers et al., 2014), as these are also likely to have occurred, although less frequently. Plastic colour preferences and confounding factors are still a debated topic with no consensus (Hidalgo-Ruz et al.,2021).

When fishing boats and trawlers do not go out into the sea, particularly during poor weather conditions or weekends (Matos et al., 2018), Yellow-legged gulls' resort to foraging alternatives. With the nearest landfill being 30 kms away (within their foraging range) from the Deserta island colony, they naturally frequent these landfills, where large quantities of anthropogenic materials including (micro)plastics are consumed (Lopes et al., 2021). Yet, during the breeding season (when the samples were collected), gulls are known to shift their diet from anthropogenic materials to a higher quality diet that mainly includes marine prey such as pelagic fish (Ramos et al., 2009), as to provide themselves and their chicks with food that better fulfils their nutritional needs (Schoener., 1971). The present findings, however, seem to suggest that landfill foraging was as prevalent as sea foraging (fishing boat interactions). This is evidenced in the regurgitates where a high prevalence of film shaped plastics (FO=39.26%) was found (when compared to the other species which did not have any films). Films are a common occurrence in landfills likely originating from transparent plastics bags. The fact that transparent coloured plastics were also found at high proportions in the faeces and regurgitates seem to further support this, as transparent plastics are also a common occurrence in landfills, originating from plastic bags or other films (GESAMP, 2016b). Furthermore, transparent plastics have been reported to have a low occurrence in pelagic areas and in species that feed on the sea surface or by diving (Hidalgo-Ruz et al., 2021). Their visits to landfills could have also contributed to the total amount of fragments found in the faeces, as fragments are also a common occurrence in landfills (Su et al., 2019; Kazour et al.,2019) thus also contributing to the total amount of fragment type of plastics found.

The low number of mesoplastics in the faeces suggests that mesoplastics are likely regurgitated due to their size as most mesoplastics cannot pass through the gastrointestinal tract of the Yellow-legged gull, thus being a possible explanation as to why the majority of the plastics found in the faeces were within the microplastic size range. It is also possible that mesoplastics further degrade due to the stomach fluids of the bird, thus getting smaller and accumulating in the faeces in the form of microplastics. Both cases showcase the superior ability of the Yellow-legged gull to regurgitate larger amounts and larger pieces of plastic. It is plausible that this ability to remove larger volumes of refuse through regurgitation, is a relative fitness-improver, that allows them to be more opportunistic. Additionally, it could also indicate that, because they consume large volumes of plastics, they might have the need to regurgitate and defecate more often, contributing to their samples being loaded with plastics. Further studies examining the relationship between faeces and regurgitates of the same individual could prove useful.

Audouin's gull. The Audouin's gull showed a high FO despite presenting a low mean number of plastics per sample. Contrary to what was expected, this species was supposed to have one of the lowest FO, and the lowest mean number of plastics, since the Audouin's gull is known to forage not far from their colonies and usually goes seawards to forage during the night (Arcos & Oro 2002). Coincidently, this species is known to mainly feed on pelagic fish found on the water column or surface, usually in pelagic areas where smaller quantities of (micro)plastics (≈0-10 items per m^3) are found, when compared to the ocean (≈100-100000 items per m^3) and intertidal sediment (≈10000-100000 items per m^3) (Hidalgo-Ruz et al., 2012). Accordingly, pelagic sea areas in the southern part of Portugal (from Lisbon to the limit of the Portuguese economic exclusive zone to the south of Portugal) are also known to have lower quantities of floating plastic debris (≈1 item per km^2) when compared to the northern area (≈2 item per km^2) (Sá et al.,2016). Furthermore, the Audouin's gull generally resorts to surface dipping to catch prey. Instead of plunging their beak into the water, this species uses their fangs to catch prey (Arcos & Oro 2002), therefore, not being susceptible to the unintentional bycatch of (micro)plastic particles in the water when foraging. Thus, trophic transfer and accumulation through the food chain might be one of the probable sources of contamination. As referenced before, trophic transfer from fish to top predators is known to occur (Carbery et al., 2018; Nelms et al., 2018). Coincidently, some species of pelagic fish that the Audouin's gull feeds on are likely exposed to (micro)plastic pollution (Renzi et al., 2019; Compa et al.,2018; Cem et al., 2019; Prata et al., 2022; da Silva et al., 2022). This exposure likely leads to the accumulation of (micro)plastics in different tissues of the fish, such as their gastrointestinal tract and areas exposed to the surrounding water such as the gills and scales (Franzellitti et al., 2019). However, the Audouin's gull diet is not exclusively composed by pelagic fish. This species has been known to also feed on epipelagic demersal fish, although less frequently (Matos et al.,2018). These fish are known to accumulate similar amounts of plastics when compared to pelagic species (see

Wootton et al., 2021 for a full literature review on the occurrences of plastics in benthic and pelagic fish), even though the former spend the majority of their time at the sediment level in shallow areas, where (micro)plastics are known to accumulate (Hidalgo-Ruz et al., 2012)., These fish could constitute another potential source of (micro)plastic intake.

Audouin's gull have also been known to interact with fishing operations. The Audouin's gull takes advantage of fishing boats to find foraging opportunities more easily (Arcos & Oros., 2002; Ouled-Cheikh et al., 2020). This likely exposes them to contamination originating from fishing gear. During purse seine (of which they are known to mainly interact with -Arcos & Oros., 2002) abrasion on the fishing nets caused by friction with sea water, is common to happen. This produces microplastics (presumed to be mostly fibres) which can bioaccumulate in the fish being caught in the net, of which the Audouin's gull opportunistically feeds on (Ouled-Cheikh et al., 2020; Napper et al.,2022). This is a likely source of the fibres found in the Audouin's gull samples. Furthermore, visits to wastewater treatment plants also expose the Audouin's gull to another potential source of fibre contamination (Matos et al., 2018). These are areas where microplastic fragments and fibres predominately accumulate (Talvitie et al., 2017). During wastewater treatment processes, fibres have been reported to decrease in amount (from the influent towards the effluent) whereas fragments increase proportionally to fibres (Talvitie et al., 2017). Even though fibres decrease in number, they are still common throughout the entire process (Talvitie et al., 2017).

The microplastic size class is the most common size found in wastewater treatment plants (Talvitie et a., 2017; Edo et al.,2020), fishing operations (Ouled-Cheikh et al., 2020; Napper et al.,2022), and epipelagic demersal fish that the Audouin's gull feed on (Hidalgo-Ruz et al., 2012; Matos et al.,2018). Coincidently, the majority of the plastics found were within the microplastic size range. This further establishes the aforementioned sources as the likely origins of the majority of the plastics found in this species. Independently of the source, it is possible that a predominant direct uptake of microplastics was the case and not a subsequent degradation of mesoplastics inside the Audouin's gull's digestive tract. If this was the case, larger pieces of microplastics were expected to be found as the degradation of mesoplastics is a gradual process. This would have contributed to significantly larger microplastics (in terms of size) in this species which would have then revealed differences in the GLMs used to compare sizes between species, however, this was not the case. Furthermore, the fact that mesoplastics were found in negligible amounts in the regurgitates (n=4, FO=3.39%) and in the faeces (n=1) further supports this idea.

Unexpectedly, brown coloured plastics were the predominant colour found on this species' samples. It is likely that trophic transfer was the main source, as surface feeding bird species have been known to have larger amounts of brown plastics when compared to other colours (Hidalgo-Ruz et al.,2021). Yet, the mechanisms behind the preferences for certain types of colours are still unknown as it is likely that this is a species-specific behaviour.

Kentish plover. A comparison of plastic ingestion data between the faecal samples of the Kentish plover (FO = 51%, n=37), and similar data from the faeces of a similar species in terms of behaviour, the common-ringed plover (*Charadrius hiaticula*) in the Tejo estuary (FO = 70%, n=30 – Lourenço et al., 2017), presented different results. These dissimilarities, although not that far apart, can be attributed to the heavy pollution of the intertidal sediments of the Tejo estuary (7.5 pieces per ml, FO=100%, n=18). The FO, shown in the present study can be attributed to the fact that most plover species, including the Kentish plover, forage in intertidal zones along the coast and in beaches. These are areas which are known accumulation hotspots for (micro)plastics (Lots et al., 2017). Coincidently, these are also areas where marine invertebrates (that the Kentish plover mainly feeds on) inhabit and forage. Therefore, it is likely that trophic transfer and accumulation could be two sources of (micro)plastic contamination in this species. Marine invertebrates (mostly filter feeders and detritivores) have been known to directly ingest plastics as they misidentify them as food items, leading to bioaccumulation in their tissues (Examples: Cole et al., 2015; Lourenço et al., 2017; Messinetti et al., 2018). Plastics ingested by these invertebrates eventually reach the Kentish plover through preying. Likewise, the Kentish plover itself can also misidentify plastic items as possible food sources (Blight et al., 1997), thus contributing as an additional source for (micro)plastic intake. Colour and size have an important role in the waterbirds ability to identify prey. Yet, as mentioned before, preferences for a certain colour are presumably species related (Hidalgo-Ruz et al., 2021).

In continental beaches, blue coloured plastics, followed by white, yellow, and red coloured plastics were the most commonly found (Hidalgo-Ruz et al.,2021). Kentish plover samples analysed in our study presented parallel results, where the majority of plastics were blue followed by red, transparent and brown. These colours were also found in other parts of the world to be prevalent in beach sediment (Tavares et al., 2020; Hidalgo-Ruz et al., 2021; Mattan-Moorgawa et al., 2021; Narciso-Ortiz et al.,2020).

In the foraging areas used by the Kentish plover, fragments were also expected to accumulate from marine debris that washes up shore or from litter scattered on the beach, that eventually decomposes into fragments due to photodegradation, wave motion and other physical or chemical processes (Cooper et al., 2012). However, beach cleaning programs have proven to be effective at removing beach litter in the Algarve region (Candeias., 2015). Transects taken along the beaches where most Kentish plovers place their nests have revealed an extremely low abundance of plastic litter (<50 pieces per transect across a 5-month span -Velez., 2017). Furthermore, since the south of Portugal is known to have lower quantities of floating marine debris, coasted beach plastics are not as common (Sá et al. 2016). The lower occurrence of plastics in the areas used by the Kentish plover ultimately contributes to a lower exposure. All of these factors are effectively mirrored, in the samples. Additionally, the fact that the majority of plastics found in this species were fibres, and predominantly microplastics, suggests that the plastics found might be originating from other sources, other than beached plastics. Some studies have suggested that these fibres might originate from wastewater that escapes estuarine systems and becomes entrapped in shoreline sediment (Browne et al., 2011). Even though estuarine systems are efficient at retaining (micro)plastics, some are still likely to pass through (Harris et al., 2003). Moreover, cloth pieces releasing fibres, like beach towels, or even airborne fibre deposition on the beaches are also likely sources (De falco et al., 2020). The former even goes in accordance with the high number of human visitors in the beaches of Algarve during the sampling period.

Notably, the Kentish plover was the species that exhibited statistically significant larger plastics when compared to the other species, this, however, did not translate into an higher amount of plastics belonging to the mesoplastic size class. This was most likely due to the high number of fibres retrieved from this species faecal samples, as fibres tend to be larger than fragments. Measuring the area of a plastic might be a more useful measure to compare between fragments and fibres, in future studies.

Little tern. Out of the 6 species, the Little tern had the lowest amount and mean number of (micro)plastics per sample. The Little tern usually surface plunges with their beaks open to catch prey, thus unintentional bycatch of particles when diving can occur and potentially be a risk factor for (micro)plastic accumulation in this species. However, plastic accumulation in the water column in both estuarine and marine environments is not common (Hidalgo-Ruz et al., 2012). The Little tern (besides in some occasions foraging in the sea), often forages in the channels of the Ria Formosa. It mainly feeds on small benthic fish (Catry et al., 2006) found at the surface and that often forage at the bottom of the lagoonar system. Because of this, larger amounts of (micro)plastics were expected to be found in the faeces, due to the expected accumulation of (micro)plastics in these fish. Likewise, fragments were also expected to be found as they are the predominant type of plastic found at the sediment level in the channels of Ria Formosa (Morgado et al.,2022).Unexpectedly, both were not the case. A possible explanation for these results can be found in the regurgitate analyses. While the faeces had low amounts of (micro)plastics when compared to the other species, the regurgitates revealed the opposite. Out of the 3 species sampled for the regurgitates, the Little tern's regurgitates was the species that showed a significantly higher occurrence and amount of overall plastics. This is further supported by the high number of plastics found in the regurgitates (n=276) and highest overall mean. Most of the pieces found were blue microplastic fragments in one of the samples, which had approximately 166 fragments. In four other samples a range of 10-33 fragments were noted. Contrastingly to the faecal samples, these findings go in accordance with the sediment sample analysis performed in the channels of the lagoon (Morgado et al., 2022), where the demersal fish that compose the majority of the Little tern's diet, mainly, feed on (Catry et al. 2006; Ramos et al.2013). Additionally, demersal fish are small in size and likely cannot ingest large pieces of plastic, therefore, accumulating plastics within the microplastic size range. Compiling this information indicates that the large number of small fragments present in the regurgitates likely reached the Little tern through trophic transfer.

The discrepancy between the amount of plastics found in the regurgitates and in the faeces can be caused by a better ability of this species to regurgitate refuse (when compared to the Audouin's gull and the Yellow-legged gull), which eventually lead to a lower accumulation of debris in the faeces. Furthermore, because only fibres were found in the faeces, it is possible that fibres more easily pass through the gastrointestinal tract of the bird, whereas fragments are more likely withheld during digestive processes and thus are regurgitated.

Black-winged stilt and Pied Avocet. These two species were expected to have large amounts and FO of (micro)plastics in their faeces. Despite both not showing a statistically significant higher prevalence, when compared to the other species, they showed some of the highest mean number of plastics per affected faecal sample. Theoretically these species would both be more frequently exposed to plastic contaminated areas. The Ria Formosa is home to 10 wastewater treatment plants whose effluents are sometimes discharged directly into the lagoon (Silva & Cravo 2020). Clandestine sewage water is also a known problem contributing to the input of anthropogenic materials into the lagoon system. It is estimated that across the region of Algarve, in southern Portugal, 10.2x10^9 to 32.0x10^9 microplastic pieces entered the environment through untreated wastewaters; and 0.3 to 0.5x10^9 entered through treated wastewater during 2019 (Prata et al 2020). Furthermore in 14 sediment samples collected across multiple sampling points in Ria Formosa, a mean abundance of 167.9 microplastics per kg of dry sediment was detected, indicating the presence of microplastics in the substrate (Morgado et al., 2022). Lagoons have also shown to be one of the most efficient systems to retain microplastics in the sediment. This is mostly due to the sediments' trapping efficiency that composes the bottom of the lagoons (Harris et al., 2003). The Black-winged stilt and Pied avocets' diet and feeding strategy along the sediment in both the channel and in salt pans, where (micro)plastics tend to accumulate (Iñiguez et al., 2017; Lourenço et al., 2017; Morgado et al., 2022), are a known risk factor for (micro)plastic contamination. Furthermore, their diets are composed of species susceptible to plastic contamination such as crustaceans, insects, and bivalves (Goriup et al., 1982). Both are also tactile foragers. The Pied avocet inserts its beak slightly open into the sediment probing for food items, inadvertently consuming any other particles, including (micro)plastics. Additionally, this species shakes its beak from side to side while in the sediment, resuspending any particles that were deposited. The Black-winged stilt has a very similar tactile foraging tactic. Instead of shaking the sediment, the Black-winged stilt pokes the sediment several times with their beaks slightly open in order to probe for food, thus also exposing them to plastic contaminants in the sediment. This species is also known to wash their prey in polluted water with resuspended sediment originating from the continued poking of the sediment.

The feeding strategies of the Black-winged stilt and Pied avocet, further explain the reason behind fibres being the predominant type of plastics found in their samples. In the Ria Formosa' sediment, the overwhelmingly majority of the plastics found on the channels were fragments (Morgado et al., 2022). Fibres were also found although at much lower proportions. The fact that the Black-winged Stilt and the Pied avocet had significantly more fibres, suggests that: (1) the prey that they consumed at the bottom of the sediment were contaminated, mostly with fibres or (2) the places where they foraged had predominantly more fibres along the sediment. However, these results contradict the sediment analysis obtained by Morgado et al., (2022), where fragments were shown to be more prevalent than fibres. The present results may be explained by the fact that both species feed mostly in saltpans and small lagoons and not as frequently on the main lagoon of Ria Formosa. Analysis of salt samples collected from saltpans in southern Spain (near Algarve) revealed a range of 100 to 150 (n=4) particles per kg of salt (0.1 - 0.15 pieces/g) (Iñiguez et al., 2017). Higher amounts have also been reported in salt pans in India where a range of 0.36 to 2.13 pieces/g of salt were reported. This higher range likely results from a higher contamination of the sampling locations (Nithin et al., 2021). Across both studies, fibres were the most common type and black, red, blue, white, and transparent, the most prevalent colours. Results from both studies coincide with the plastics found in the faecal samples of both species where, fibres were the predominant type and blue, transparent, and red were common occurrences and found in relatively high proportions.

The fact that both species mostly feed in salt pans could indicate that the Pied avocet and the Black-winged stilt faecal samples do not efficiently mirror the type nor quantity of plastics present in the sediments of the lagoon but might, however, better reflect the contamination in salt pans. There are also seasonal and anthropogenic changes and factors such as rainfall rate, turbidity, water currents or wastewater effluents dumps that influence the presence of certain types and quantities of plastics on the sediments of the channel and in the salt pans (Kazour et al., 2019; Sousa et al., 2021; Nithin et al., 2021). Effluent dumps from wastewater treatment plants, might be especially relevant since they mostly contain microplastic fragments and fibres (Talvitie et al., 2017; Kazour et al., 2019). These microplastics eventually deposit into the sediment through biofouling and biofilm forming processes (Kaiser et al., 2017), eventually influencing the type, quantity and size class of plastics found in the samples of waterbird species that feed along the sediment. Furthermore, it is possible that abandoned salt pans (where most of both species' individuals inhabit and forage) function as efficient traps for plastics, since the water inside the pans is not often renewed. Further airborne deposition of fibres originating from human activities nearby could exacerbate the amounts of fibres accumulated in salt pans' sediments, thus exposing the Black-winged stilt and the Pied avocet to heavily contaminated environments.

4.2 - Additives in eggs

The correlation between the number of plastics found in the faeces and the amount of additives in the eggs was not significant. A major contributor to this result is likely the fact that faecal samples collected did not belong to the same individuals that laid the eggs. The intended goal, however, was to have a population overview of these contaminants, and not an individual perspective. Since no correlation was found it suggests that the sample size might not have been big enough (n=10 per species) to make such a correlation at the population level. However, collecting more eggs during the reproductive season poses a serious ethical challenge.

Even though the correlation could not be established, a first evaluation of the exposure of waterbirds to brominated flame retardants using eggs is reported. There are many factors influencing the amounts and proportions of the PBDEs found, some of which are still yet unknown. However, it is known that salinity does not appear to be a significant factor influencing the leaching of organic additives such as PBDEs (Capolupo et al., 2020), suggesting that there are other factors at play. While it is true that waterbirds might be exposed to additive contamination through the direct consumption of contaminated water; through the release of these contaminants after ingestion of plastics due to the birds' stomach oils (Tanaka et al., 2015); atmospheric and dermal exposure, as well as, the consumption of contaminated prey, there are further factors influencing the total concentrations and proportions of PBDEs (Darnerud et al., 2003). Atmospheric exposure of the eggs could have also been an additional factor contributing to the present results (Gouin & Harner 2003).

Species that feed along the sediment were expected to have larger concentrations of PBDE. Heavier PBDEs, (i.e with more bromide ions), tend to be denser and sink, eventually depositing and accumulating in benthic sediments and in bottom dwelling organisms (Palm et al., 2002; Gouin & Harner, 2003). Hence, species that feed along the sediment (Pied avocet and Black-winged stilt) were expected to have larger cumulative concentrations as well as higher proportions of heavier PBDEs (BDE. 153, 154 and 183). By contrast, species that feed on pelagic areas (Audouin's gull and Little tern) and transition areas (Kentish plover) were expected to have lower cumulative concentrations and larger proportions of lighter PBDEs (BDE 28, 47 and 99). Yet, this was not the case since the Pied avocet had the lowest cumulative concentrations of PBDEs followed by the Kentish plover, whereas, by contrast, the Yellow-legged gull consumed the highest cumulative concentration which goes in accordance with their interactions with landfills and other heavily impacted areas (Lopes et al.,2021). The remaining species revealed similar amounts and proportions of the chemicals.

BDE28 seemed to be the dominant type of additive across all species. This was not expected as environmental studies performed, revealed that BDE28 was one of the less commonly found compounds (Wang et al., 2007) due to its low half-life (3 orders of magnitude lower when compared to other PBDEs) in the environment (Hale et al.,2003; Gouin & Harner.,2003). By contrast heavier PBDEs (BDE 153, 154 and 183) have a much longer half-life in the environment, taking longer to degrade and thus being more prevalent (Palm et al., 2002). Therefore, it is possible that the metabolism of each species progressively deteriorated these heavier PBDEs into less brominated particles. Since BDE28 was the least brominated PBDE examined, it makes sense that it was the compound with the largest relative proportion. This is further evidenced as the heavier PBDEs are generally in a declining order (BDE183<BDE99<BDE47<BDE28). Metabolism and elimination rates of these compounds are species-specific, therefore, using eggs as potential proxies may not reflect the real environmental concentrations. However, they can provide information in the quantity (concentrations) and prevalence of these compounds in waterbird species.

Similar total PBDE concentrations across the species suggest a widespread distribution of these contaminants among sea and estuarine environments., However, they appear to be at least 2 to 3 orders of magnitude below the LD50 threshold on mammals of 2 500 000 ng/500mg (5000 ng/mg) for PentaBDE (BDE 47, 99, 153, 154) and 1 000 000 ng/500mg (2000ng/mg) for OctaBDE (BDE183 (IPCS, 1994a; Darnerud ., 2003). Therefore, it is also likely that the threshold for these waterbirds was not reached. However, since few studies have been performed to date on the impact of these contaminants on waterbirds it is not possible to ascertain with complete certainty what concentrations can be considered deleterious for the species of birds sampled in this study, especially in embryos (as a consequence of long periods of exposure). Further environmental analysis and ecotoxicological studies for these contaminants in southern Portugal are needed.

4.3 - Overall

These findings ultimately show that due to a lack of significant differences on the frequency of occurrence of plastics across the studied bird species, a contamination gradient from the estuary towards the ocean cannot be completely confirmed. However, since there are decreasing frequencies of occurrence and amounts along the gradient in both faecal and regurgitate samples, this hypothesis cannot be completely discarded.

Plastic fibres seemed to be more prevalent in the faeces, with a significantly higher amount in the faecal samples of the species that predominantly feed in the salt pans (Black-winged stilt and Pied avocet), where, theoretically, there is a higher exposure. Large amounts of fragments in the Little tern's regurgitates were noted. These go in accordance with the sediment sample analysis of the channels of the lagoon where the majority of the Little tern's prey forage. The Kentish plover was not as exposed to environmental contamination due to beach cleaning programs and low pelagic plastic pollution, however, their prey likely is. Notably, the Audouin's gull also had unexpectedly high amounts of fibres, although most likely originating from their interactions with fishing operations. Fragments and films were predominant in the Yellow-legged gull mostly due to their visits to heavily impacted areas such as landfills. Compiling these findings ultimately shows that these species approximately reflected the environmental contaminations found in previous studies and in the environments exploited by them.

Plastic colours found in the faeces and regurgitates revealed no notable trend across the gradient. The most common colours found across the species were coincidently found to be present at the sediment level in the channels of Ria Formosa (Morgado et al., 2022) and are relatively abundant in seawater surface (first 3m – Lusher et al., 2014), in salt pans (Iñiguez et al., 2017; Nithin et al., 2021) and in beaches (Hidalgo-Ruz et al., 2021). A mix of colours in varying percentages of occurrence and amounts seem to be the case for most species. This is further evidenced in the NMDS analysis presented where samples were not segregated. Rather, a prevalence of blue, transparent, red, and brown coloured plastics seems to be the case.

No relevant trend across the gradient was found for the size of plastics. The majority were microplastics, with a few exceptions. This could be explained by the following non-mutually exclusive reasons: (a) the birds having efficient mechanisms of plastic degradation after ingestion, (b) there is a predominantly higher prevalence of microplastic contamination in the environment, or (c) the main route of exposure to plastic contamination is via trophic transfer (i.e., diet).

Additives also revealed no significant trend across the gradient. The high proportions of BDE28 were most likely due to the degradation into lower brominated congeners in the environment and/or after ingestion.

4.4 - Waterbirds as sentinels in Ria Formosa

Waterbird species have the ability of becoming possible sentinel species for contaminants. In the north Atlantic and North-eastern pacific, Northern Fulmars (*Fulmarus glacialis*) are now used as a sentinel of plastic pollution (Avery-Gomm et al., 2012). Due to their large foraging areas, they provide a wholistic view of the geographical region. Their carcasses and stomach samples are used as a proxy of plastic pollution. Using this species as a base model, a good sentinel species should fulfil the following important conditions: (1) their diet should resemble the sensitive species to monitor; (2) it should cover the intended area to monitor; (3) be abundant and easy to find; (4) easy to sample; (5) susceptible to the type of pollution intended to monitor. The species sampled in our study fulfil most of these requisites, however, because Ria Formosa is important as a nursery of marine organisms, including those for human consumption (see Abecasis., 2008, 2009; Correia et al., 2015), a species that inhabits this area concurrently with the species intended to monitor, and that exploits most of the lagoons' habitats (salt pans, beaches, small ponds etc…) should be considered. It is important to note, that details from the area intended to study have a large influence in the species selected to monitor it. Definitively selecting a species to be a sentinel, is only possible through cross-examination between results of analysis obtained from faeces and regurgitates and deep ecological knowledge of the species in the area intended to study (obtained from behavioural, tracking and diet analysis). However, the selection of a sentinel species to monitor a certain area can only be achieved if preliminary studies evaluating a variety of species and, whether or not (and to what extent), species are exposed to a certain contaminant, such as the present, are performed. Therefore, since the intended area to monitor focuses in the Ria Formosa, and its ecosystem service providing, the Little tern, Black-winged stilt and the Pied avocet were selected as potentially good candidates. It is important to note that even though the Yellow-legged gull, Audouin's gull and Kentish plover were not selected it does not mean that they might not be useful indicators in other areas, it only implies that they might not reflect plastic or additive pollution in the intended area to study.

Using the Little tern could prove useful as it is the only species that almost exclusively feeds on the lagoon channels. Data from this species faeces and regurgitates, could, therefore, complement data obtained from the Black-winged Stilt and Pied avocet which mainly forage in saltpans and small lagoons. These three species are also easy to sample, commonly found and have a wide distribution around Ria Formosa, hence, using them in conjunction could offer a wholistic view of spatial and temporal trends of plastics and additives pollution across the entirety of the Ria Formosa. Besides, the suggested species are directly and/or indirectly exposed to (micro)plastic and additive contamination. They might ingest these pollutants (albeit most of the times unintentionally) directly from the environment, or indirectly through trophic transfer across the food web. Additionally, the Black-winged stilt and the Pied avocet had a high prevalence and number of plastics across their samples, comparable to data from blue mussels sampled in various sites in the Algarve region (Vital et al., 2021).

Despite their potential use as good sentinels, different types of samples should also be considered, especially since faeces and regurgitates only offer a limited timeframe view of the pollutants consumed. Physiological measures reflecting body function, developmental measures and fertility would be an important complement to the sampling methods employed in our study, revealing at the same time potential underlying detrimental effects of both additives and (micro)plastic ingestion on the health status and fitness of these species.

4.5 - Conclusion

In order for proper legislation to be applied, environmental assessment and monitoring programs need to be implemented to better understand (micro)plastic and additive pollution challenges and trends. Identifying contaminated areas and their extent; identifying plastic distribution and factors influencing exposure and identifying affected species and factors influencing their uptake are some of the imposed challenges. However, current knowledge gaps do not allow us to answer most of these challenges. This thesis tried to bridge some of these knowledge gaps by presenting a first descriptive analysis of plastics found in faeces and regurgitates of 6 waterbird species in southern

Portugal. The majority of the (micro)plastics catalogued across the faeces and the regurgitates of the species were microplastics, mainly fibres and fragments. A large proportion were blue, followed by transparent, red and brown. Analysis of the polymers resorting to µ-FTIR was not a possibility due to time constraints but would be useful to ascertain the origin of these plastics. No correlation was found between the number of plastics consumed and the additives assimilated in the eggs. However, all target PBDE were found in the eggs of every sampled species, both, in similar concentrations and proportions, indicating their prevalence in coastal, estuarine environments, as well as, in top predators. Furthermore, a gradient of (micro)plastic contamination along an estuarine-sea gradient could not be firmly established. Yet, the declining frequencies of occurrence and quantities of overall (micro)plastics found in the samples, suggest that this gradient cannot be completely excluded. Comparing (micro)plastic content in the faeces of species that forage more inland and those that forage in transition zones or nearer to the seashore, has shown that there is indeed a downward trend. Likewise comparing the regurgitates of a species that is coastal with those that have pelagic habits has shown similar results. The (unexpected) large amount of (micro)plastics found in some of the species reveal that similar to the additives, plastic pollution is now widespread, suggesting a growing pervasiveness in coastal and estuarine environments.

Considering the intrinsic characteristics of the different species when evaluating (micro)plastic and additive contamination, allowed us to take a first step towards the identification of possible species that, in the future, can be used as efficient sentinels to assess, some of the still unknown, spatial and temporal trends of (micro)plastic and additive pollution in coastal and estuarine environments. However, to ultimately identify a proper sentinel species to monitor these environments, further contamination studies need to be developed using the suggested candidate species (Little tern, Black-winged stilt and the Pied avocet). Studies to validate the data presented in this thesis, to identify which polymers are more persistent in these individuals (and if there is a trend), and to further contribute to their establishment as sentinel species, are needed. Only by doing this, will it be possible, in the future, to assess spatial and temporal trends in plastic and additive contamination in coastal and estuarine environments.

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Supplementary Material

Table S1 – Binomial matrixes used to perform the frequency of occurrence calculations and binomial GLM for the faecal samples. Presence of a given category is marked by the number 1 and absence by the number 0. Species presented are listed as follows: HH – Black-winged stilt, RA – Pied avocet, CA – Kentish plover, SA – Little tern, IA – Audouin's gull, LM – Yellow-legged gull. Continuation of the table is shown in the next pages.

ID	Species	Faeces	Fragment	Fibre	Filament	Sfere	Film	Ball	Blue	Green	Black	Purple	Transpare	White	Yellow	Red	Grey	Pink	Brown	Multicolo	Mesoplast	LMP	SMPP	NP
174	SA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
173	SA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
172	SA	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0
171	SA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
170	SA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
169	SA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
168	SA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
167	SA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
166	SA	1	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0
165	SA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
164	SA	1	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0
163	SA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
162	SA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
161	SA	1	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
160	SA	1	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0
159	SA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
158	SA	1	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
157	SA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
156	SA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
155	SA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
154	SA	1	0	1	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	1	1	0
153	SA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
152	SA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
151	SA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
150	SA	1	0	1	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	1	1	0
149	SA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
148	SA	1	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
147	SA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
146	SA	1	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
145	SA	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0
144	SA	1	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0

ID	Species	Faeces	Fragment	Fibre	Filament	Sfere	Film	Ball	Blue	Green	Black	Purple	Transpare	White	Yellow	Red	Grey	Pink	Brown	Multicolo	Mesoplas	LMP	SMPP	NP
143	RA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
142	RA	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0
141	RA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
140	RA	1	0	1	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0
139	RA	1	0	1	0	0	0	0	1	0	0	0	1	0	0	0	0	0	1	0	0	0	1	0
138	RA	1	0	1	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0
137	RA	1	1	1	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	1	1	0
136	RA	1	1	1	0	0	0	0	1	0	0	1	0	0	0	1	0	0	0	0	0	1	1	0
135	RA	1	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0
134	RA	1	1	1	0	0	0	0	0	0	0	0	1	0	0	1	0	0	1	0	1	1	0	0
133	RA	1	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0
132	RA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
131	RA	1	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
130	RA	1	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
129	RA	1	0	1	0	0	0	0	1	0	0	0	1	0	0	0	0	0	1	0	0	1	0	0
128	RA	1	0	1	0	0	0	0	1	0	0	0	1	0	0	1	0	0	1	0	0	1	1	0
127	RA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
126	RA	1	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0
125	RA	1	0	1	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0
124	RA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
123	RA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
122	RA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
121	RA	1	0	1	0	0	0	0	1	0	0	0	1	0	0	1	0	0	1	0	0	1	1	0
120	RA	1	0	1	0	0	0	0	1	0	1	0	0	0	0	1	0	0	1	0	0	1	1	0
119	RA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
118	RA	1	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	1	1	0
117	RA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
116	RA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
115	RA	1	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0
114	RA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
113	RA	1	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0
112	RA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
111	RA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

ID	Species	Faeces	Fragment	Fibre	Filament	Sfere	Film	Ball	Blue	Green	Black	Purple	Transpare	White	Yellow	Red	Grey	Pink	Brown	Multicolo	Mesoplas	LMP	SMPP	NP
110	LM	1	0	1	0	0	0	0	1	0	0	0	1	0	0	0	0	0	1	0	0	1	1	0
109	LM	1	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
108	LM	1	0	1	0	0	0	0	1	0	0	0	1	0	0	0	0	0	1	0	1	1	1	0
107	LM	1	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0
106	LM	1	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
105	LM	1	0	1	0	0	0	0	1	0	0	0	1	1	0	1	0	0	1	0	1	1	1	0
104	LM	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
103	LM	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
102	LM	1	0	1	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	1	1	1	0
101	LM	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
100	LM	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
99	LM	1	1	1	0	0	0	1	1	1	1	1	1	1	0	0	0	0	0	0	0	1	1	1
98	LM	1	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0
97	LM	1	0	1	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	1	0	1	0	0
96	LM	1	0	1	0	0	0	0	1	0	1	0	1	0	0	0	0	0	0	0	0	1	1	0
95	LM	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
94	LM	1	0	1	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0
93	LM	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
92	LM	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
91	LM	1	1	1	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	1	1	1	0
90	LM	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
89	LM	1	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	1	1	0
88	LM	1	0	1	0	0	0	0	1	0	1	0	1	0	0	1	0	0	0	0	0	1	0	0
87	LM	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
86	LM	1	1	1	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	1	1
85	LM	1	0	1	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	1	1	0
84	LM	1	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
83	LM	1	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
82	LM	1	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0
81	LM	1	1	1	0	0	0	0	1	0	0	0	1	1	0	0	0	0	0	0	0	1	1	0
80	LM	1	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0
79	LM	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

ID	Species	Faeces	Fragment	Fibre	Filament	Sfere	Film	Ball	Blue	Green	Black	Purple	Transpare	White	Yellow	Red	Grey	Pink	Brown	Multicolo	Mesoplas	LMP	SMPP	NP
78	IA	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0
77	IA	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0
76	IA	1	0	1	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0
75	IA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
74	IA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
73	IA	1	0	1	0	0	0	0	1	0	0	0	1	0	0	0	0	0	1	0	1	1	0	0
72	IA	1	1	1	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	1	1	0
71	IA	1	1	1	0	0	0	0	1	0	1	0	1	0	0	1	0	0	1	0	0	1	1	0
70	IA	1	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0
69	IA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
68	IA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
67	IA	1	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0
66	IA	1	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0
65	IA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
64	IA	1	1	1	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	1	1	0
63	IA	1	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0
62	IA	1	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
61	IA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
60	IA	1	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
59	IA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
58	IA	1	0	1	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	1	0	0
57	IA	1	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
56	IA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
55	IA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
54	IA	1	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
53	IA	1	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0
52	IA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
51	IA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
50	IA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
49	IA	1	1	1	0	0	0	0	1	0	0	0	0	0	0	1	0	0	1	0	0	1	1	0
48	IA	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0
47	IA	1	1	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	1	1	0

ID	Species	Faeces	Fragment	Fibre	Filament	Sfere	Film	Ball	Blue	Green	Black	Purple	Transpare	White	Yellow	Red	Grey	Pink	Brown	Multicolo	Mesoplast	LMP	SMPP	NP
46	HH	1	0	1	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	1	1	0
45	HH	1	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	1	1	0	0
44	HH	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
43	HH	1	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0
42	HH	1	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0
41	HH	1	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0
40	HH	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
39	HH	1	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0

ID	Species	Faeces	Fragment	Fibre	Filament	Sfere	Film	Ball	Blue	Green	Black	Purple	Transpare	White	Yellow	Red	Grey	Pink	Brown	Multicolo	Mesoplast	LMP	SMPP	NP
38	CA	1	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
37	CA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
36	CA	1	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
35	CA	1	1	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0
34	CA	1	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
33	CA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
31	CA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
30	CA	1	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0
29	CA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
28	CA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
27	CA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
26	CA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
25	CA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
24	CA	1	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
23	CA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
22	CA	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0
21	CA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
20	CA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
19	CA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
18	CA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
17	CA	1	1	1	0	0	0	0	1	0	0	0	1	0	0	1	0	0	0	0	1	1	0	0
16	CA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
15	CA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
14	CA	1	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
13	CA	1	1	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0
12	CA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
11	CA	1	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0
10	CA	1	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
9	CA	1	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0
8	CA	1	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
7	CA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
6	CA	1	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
5	CA	1	0	1	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	1	1	0
4	CA	1	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0
3	CA	1	0	1	0	0	0	1	0	0	0	0	1	0	0	1	0	0	0	0	0	1	0	0
2	CA	1	0	1	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	1	1	0
1	CA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Table S2 – Binomial matrixes used to perform the frequency of occurrence calculations and binomial GLM for the regurgitate samples. Presence of a given category is represented by the number 1 and absence by the number 0. Species presented are listed as follows: SA – Little tern, IA – Audouin's gull, LM – Yellow-legged gull. Continuation of the table is shown in the next pages.

ID	Species	Faeces	Fragment	Fibre	Filament	Sfere	Film	Ball	Blue	Green	Black	Purple	Transpare	White	Yellow	Red	Grey	Pink	Brown	Multicolo	Mesoplast	LMP	SMPP	NP
22	SA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
23	SA	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0
24	SA	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0
25	SA	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0
26	SA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
27	SA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
28	SA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
30	SA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
30	SA	1	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
31	SA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
32	SA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
32	SA	1	1	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	1	1	0
33	SA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
33	SA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
34	SA	1	1	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0
36	SA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
37	SA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
38	SA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
39	SA	1	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
43	SA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
43	SA	1	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0
44	SA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
45	SA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
46	SA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
48	SA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
54	SA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
54	SA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
55	SA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
55	SA	1	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
56	SA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
57	SA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
69	SA	1	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
78	SA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
83	SA	1	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
84	SA	1	1	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0
84	SA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
85	SA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
87	SA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
88	SA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
89	SA	1	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
90	SA	1	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0
93	SA	1	1	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0
94	SA	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0
95	SA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
96	SA	1	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
98	SA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
98	SA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
108	SA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
110	SA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
116	SA	1	0	1	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0
117	SA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
125	SA	1	0	1	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	1	1	0
128	SA	1	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0

ID	Species	Faeces	Fragment	Fibre	Filament	Sfere	Film	Ball	Blue	Green	Black	Purple	Transpare	White	Yellow	Red	Grey	Pink	Brown	Multicolo	Mesoplas	LMP	SMPP	NP
100	LM	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
101	LM	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
102	LM	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
103	LM	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
104	LM	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
105	LM	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
106	LM	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
107	LM	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
108	LM	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
109	LM	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
110	LM	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
111	LM	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
112	LM	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
113	LM	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
114	LM	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
115	LM	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
116	LM	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
117	LM	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
118	LM	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
119	LM	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
120	LM	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
121	LM	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
122	LM	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
123	LM	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
124	LM	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
125	LM	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
126	LM	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
127	LM	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
128	LM	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
129	LM	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
130	LM	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
131	LM	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
132	LM	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
133	LM	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
134	LM	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
135	LM	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
136	LM	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
137	LM	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
75	LM	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
76	LM	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
77	LM	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
78	LM	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
79	LM	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
80	LM	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
81	LM	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
82	LM	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
83	LM	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
84	LM	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
85	LM	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
86	LM	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
87	LM	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
88	LM	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
89	LM	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
90	LM	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
91	LM	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
92	LM	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
93	LM	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
94	LM	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
95	LM	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
96	LM	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
97	LM	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
98	LM	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
99	LM	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

ID	Species	Faeces	Fragment	Fibre	Filament	Sfere	Film	Ball	Blue	Green	Black	Purple	Transpare	White	Yellow	Red	Grey	Pink	Brown	Multicolo	Mesoplast	LMP	SMPP	NP
17	IA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
18	IA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
19	IA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
20	IA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
21	IA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
22	IA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
23	IA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
24	IA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
25	IA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
26	IA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
27	IA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
28	IA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
29	IA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
30	IA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
31	IA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
32	IA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
33	IA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
34	IA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
35	IA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
36	IA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
37	IA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
38	IA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
39	IA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
40	IA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
41	IA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
42	IA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
43	IA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
44	IA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
45	IA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
46	IA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
47	IA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
48	IA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
49	IA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
50	IA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
51	IA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
52	IA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
53	IA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
54	IA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
55	IA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
56	IA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
57	IA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
58	IA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
59	IA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
60	IA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
61	IA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
62	IA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
63	IA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
64	IA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
65	IA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
66	IA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
67	IA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
68	IA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
69	IA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
70	IA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
71	IA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
72	IA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
73	IA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Table S3 – Matrix used to calculate the relative proportions in the faecal samples. Data also used for the Poisson and zero inflated GLMs. Total number of plastics found and their categories are shown. Species presented are listed as follows: HH – Black-winged stilt, RA – Pied avocet, CA – Kentish plover, SA – Little tern, IA – Audouin's gull, LM – Yellow-legged gull. Continuation of the table is shown in the next pages.

ID	Species	Faeces	Total	Fragment	Fibre	Filament	Sfere	Film	Ball	Blue	Green	Black	Purple	Transparent	White	Yellow	Red	Grey	Pink	Brown	Multicolour	Mesoplastic	LMP	SMPP	NP
SA-174	SA	174	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
SA-173	SA	173	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
SA-172	SA	172	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0
SA-171	SA	171	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
SA-170	SA	170	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
SA-169	SA	169	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
SA-168	SA	168	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
SA-167	SA	167	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
SA-166	SA	166	1	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0
SA-165	SA	165	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
SA-164	SA	164	1	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0
SA-163	SA	163	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
SA-162	SA	162	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
SA-161	SA	161	1	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
SA-160	SA	160	1	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
SA-159	SA	159	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
SA-158	SA	158	1	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
SA-157	SA	157	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
SA-156	SA	156	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
SA-155	SA	155	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
SA-154	SA	154	2	0	2	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	1	1	0
SA-153	SA	153	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
SA-152	SA	152	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
SA-151	SA	151	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
SA-150	SA	150	4	0	4	0	0	0	0	0	0	0	0	3	1	0	0	0	0	0	0	0	1	3	0
SA-149	SA	149	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
SA-148	SA	148	1	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
SA-147	SA	147	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
SA-146	SA	146	2	0	2	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0
SA-145	SA	145	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0
SA-144	SA	144	1	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0

ID	Species	Faeces	Total	Fragment	Fibre	Filament	Sfere	Film	Ball	Blue	Green	Black	Purple	Transpare	White	Yellow	Red	Grey	Pink	Brown	Multicolo	Mesoplast	LMP	SMPP	NP
RA-143	RA	143	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
RA-142	RA	142	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0
RA-141	RA	141	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
RA-140	RA	140	2	0	2	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	2	0
RA-139	RA	139	3	0	3	0	0	0	0	1	0	0	0	1	0	0	0	0	0	1	0	0	0	3	0
RA-138	RA	138	2	0	2	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	2	0
RA-137	RA	137	1	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0
RA-136	RA	136	6	1	5	0	0	0	0	4	0	0	1	0	0	0	1	0	0	0	0	0	4	2	0
RA-135	RA	135	1	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0
RA-134	RA	134	2	1	1	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	1	1	0	0
RA-133	RA	133	1	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0
RA-132	RA	132	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
RA-131	RA	131	3	3	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0
RA-130	RA	130	1	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
RA-129	RA	129	3	0	3	0	0	0	0	1	0	0	0	2	0	0	0	0	0	0	0	0	2	1	0
RA-128	RA	128	7	0	7	0	0	0	0	1	0	0	0	4	0	0	1	0	0	1	0	0	6	1	0
RA-127	RA	127	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
RA-126	RA	126	1	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0
RA-125	RA	125	2	0	2	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	2	0	0
RA-124	RA	124	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
RA-123	RA	123	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
RA-122	RA	122	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
RA-121	RA	121	14	0	14	0	0	0	0	6	0	0	0	5	0	0	1	0	0	2	0	0	10	4	0
RA-120	RA	120	5	0	5	0	0	0	0	2	0	1	0	0	0	0	1	0	0	1	0	0	2	3	0
RA-119	RA	119	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
RA-118	RA	118	3	0	3	0	0	0	0	2	0	0	0	0	0	0	0	0	0	1	0	0	2	1	0
RA-117	RA	117	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
RA-116	RA	116	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
RA-115	RA	115	2	0	2	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0
RA-114	RA	114	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
RA-113	RA	113	2	0	2	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	1	1	0
RA-112	RA	112	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
RA-111	RA	111	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

ID	Species	Faeces	Total	Fragment	Fibre	Filament	Sfere	Film	Ball	Blue	Green	Black	Purple	Transpare	White	Yellow	Red	Grey	Pink	Brown	Multicolo	Mesoplas	LMP	SMPP	NP
LM-110	LM	110	4	0	4	0	0	0	0	1	0	0	0	1	0	0	0	0	0	2	0	0	2	2	0
LM-109	LM	109	3	0	3	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0
LM-108	LM	108	14	0	14	0	0	0	0	1	0	0	0	12	0	0	0	0	0	1	0	2	9	3	0
LM-107	LM	107	7	0	7	0	0	0	0	0	0	0	0	7	0	0	0	0	0	0	0	0	7	0	0
LM-106	LM	106	1	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
LM-105	LM	105	10	0	10	0	0	0	0	1	0	0	0	5	1	0	2	0	0	1	0	1	5	4	0
LM-104	LM	104	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
LM-103	LM	103	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
LM-102	LM	102	6	0	6	0	0	0	0	1	0	0	0	5	0	0	0	0	0	0	0	2	1	3	0
LM-101	LM	101	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
LM-100	LM	100	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
LM-99	LM	99	14	11	2	0	0	0	1	6	2	2	1	1	2	0	0	0	0	0	0	0	2	11	1
LM-98	LM	98	3	0	3	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	3	0	0
LM-97	LM	97	3	0	3	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	1	0	3	0	0
LM-96	LM	96	9	0	9	0	0	0	0	3	0	1	0	5	0	0	0	0	0	0	0	0	6	3	0
LM-95	LM	95	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
LM-94	LM	94	3	0	3	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	3	0	0
LM-93	LM	93	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
LM-92	LM	92	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
LM-91	LM	91	15	1	14	0	0	0	0	3	0	0	0	12	0	0	0	0	0	0	0	3	9	3	0
LM-90	LM	90	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
LM-89	LM	89	4	0	4	0	0	0	0	0	0	0	0	4	0	0	0	0	0	0	0	1	2	1	0
LM-88	LM	88	5	0	5	0	0	0	0	1	0	1	0	1	0	0	2	0	0	0	0	0	5	0	0
LM-87	LM	87	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
LM-86	LM	86	6	5	1	0	0	0	0	5	0	1	0	0	0	0	0	0	0	0	0	0	0	1	5
LM-85	LM	85	5	0	5	0	0	0	0	2	0	0	0	3	0	0	0	0	0	0	0	0	4	1	0
LM-84	LM	84	1	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
LM-83	LM	83	2	2	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0
LM-82	LM	82	1	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0
LM-81	LM	81	15	1	14	0	0	0	0	3	0	0	0	10	2	0	0	0	0	0	0	0	8	7	0
LM-80	LM	80	2	0	2	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	2	0	0
LM-79	LM	79	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

ID	Species	Faeces	Total	Fragment	Fibre	Filament	Sfere	Film	Ball	Blue	Green	Black	Purple	Transpare	White	Yellow	Red	Grey	Pink	Brown	Multicolo	Mesoplast	LMP	SMPP	NP
IA-78	IA	78	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0
IA-77	IA	77	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0
IA-76	IA	76	2	0	2	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	2	0	0
IA-75	IA	75	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
IA-74	IA	74	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
IA-73	IA	73	3	0	3	0	0	0	0	1	0	0	0	1	0	0	0	0	0	1	0	1	2	0	0
IA-72	IA	72	2	1	1	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	1	1	0
IA-71	IA	71	6	0	6	0	0	0	0	1	0	1	0	2	0	0	1	0	0	1	0	0	5	1	0
IA-70	IA	70	1	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0
IA-69	IA	69	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
IA-68	IA	68	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
IA-67	IA	67	3	0	3	0	0	0	0	2	0	0	0	0	0	0	0	0	0	1	0	0	0	3	0
IA-66	IA	66	1	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0
IA-65	IA	65	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
IA-64	IA	64	3	1	2	0	0	0	0	2	0	0	0	1	0	0	0	0	0	0	0	0	1	2	0
IA-63	IA	63	1	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0
IA-62	IA	62	1	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
IA-61	IA	61	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
IA-60	IA	60	1	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
IA-59	IA	59	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
IA-58	IA	58	3	0	3	0	0	0	0	0	0	0	0	2	0	0	1	0	0	0	0	0	3	0	0
IA-57	IA	57	1	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
IA-56	IA	56	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
IA-55	IA	55	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
IA-54	IA	54	1	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
IA-53	IA	53	2	0	2	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	2	0	0
IA-52	IA	52	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
IA-51	IA	51	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
IA-50	IA	50	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
IA-49	IA	49	3	1	2	0	0	0	0	1	0	0	0	0	0	0	1	0	0	1	0	0	2	1	0
IA-48	IA	48	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0
IA-47	IA	47	2	1	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	1	1	0

ID	Species	Faeces	Total	Fragment	Fibre	Filament	Sfere	Film	Ball	Blue	Green	Black	Purple	Transpare	White	Yellow	Red	Grey	Pink	Brown	Multicolou	Mesoplast	LMP	SMPP	NP
HH-46	HH	46	7	0	7	0	0	0	0	6	0	0	0	1	0	0	0	0	0	0	0	0	5	2	0
HH-45	HH	45	3	0	3	0	0	0	0	2	0	0	0	0	0	0	0	0	0	1	0	1	2	0	0
HH-44	HH	44	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
HH-43	HH	43	1	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0
HH-42	HH	42	3	0	3	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	2	1	0
HH-41	HH	41	1	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0
HH-40	HH	40	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
HH-39	HH	39	3	0	3	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	2	1	0

ID	Species	Faeces	Total	Fragment	Fibre	Filament	Sfere	Film	Ball	Blue	Green	Black	Purple	Transpare	White	Yellow	Red	Grey	Pink	Brown	Multicolo	Mesoplast	LMP	SMPP	NP
CA-38	CA	38	1	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
CA-37	CA	37	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
CA-36	CA	36	1	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
CA-35	CA	35	4	1	3	0	0	0	0	4	0	0	0	0	0	0	0	0	0	0	0	0	3	1	0
CA-34	CA	34	2	0	2	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0
CA-33	CA	33	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
CA-31	CA	31	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
CA-30	CA	30	2	0	2	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0
CA-29	CA	29	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
CA-28	CA	28	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
CA-27	CA	27	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
CA-26	CA	26	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
CA-25	CA	25	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
CA-24	CA	24	1	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
CA-23	CA	23	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
CA-22	CA	22	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0
CA-21	CA	21	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
CA-20	CA	20	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
CA-19	CA	19	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
CA-18	CA	18	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
CA-17	CA	17	3	1	2	0	0	0	0	1	0	0	0	1	0	0	1	0	0	0	0	1	2	0	0
CA-16	CA	16	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
CA-15	CA	15	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
CA-14	CA	14	1	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
CA-13	CA	13	3	2	1	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	1	2	0
CA-12	CA	12	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
CA-11	CA	11	1	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
CA-10	CA	10	1	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
CA-9	CA	9	2	1	1	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	2	0
CA-8	CA	8	2	0	2	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	2	0
CA-7	CA	7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
CA-6	CA	6	1	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
CA-5	CA	5	7	0	7	0	0	0	0	5	0	0	0	0	0	0	2	0	0	0	0	0	5	2	0
CA-4	CA	4	1	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0
CA-3	CA	3	2	0	1	0	0	0	1	0	0	0	0	1	0	0	1	0	0	0	0	0	2	0	0
CA-2	CA	2	2	0	2	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	1	1	0
CA-1	CA	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Table S4 – Matrix used to calculate the relative proportions in the regurgitates. Data also used for the poisson and zero inflated GLM. Total number of plastics found, and their categories are shown. Species presented are listed as follows: SA – Little tern, IA – Audouin's gull, LM – Yellow-legged gull. Continuation of the table is shown in the next pages.

ID	Species	Faeces	Total	Fragment	Fibre	Filament	Sfere	Film	Ball	Blue	Green	Black	Purple	Transparent	White	Yellow	Red	Grey	Pink	Brown	Multicolour	Mesoplastic	LMP	SMPP	NP
SA-84	SA	84	166	165	1	0	0	0	0	166	0	0	0	0	0	0	0	0	0	0	0	0	1	165	0
SA-96	SA	96	33	33	0	0	0	0	0	33	0	0	0	0	0	0	0	0	0	0	0	0	0	33	0
SA-89	SA	89	18	18	0	0	0	0	0	18	0	0	0	0	0	0	0	0	0	0	0	0	0	18	0
SA-32	SA	32	10	9	1	0	0	0	0	9	0	0	0	0	0	0	0	0	0	1	0	0	1	9	0
SA-1	SA	1	4	0	4	0	0	0	0	2	0	0	2	0	0	0	0	0	0	0	0	0	3	1	0
SA-128	SA	128	3	0	3	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	2	1	0
SA-34	SA	34	3	1	2	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	2	1	0
SA-4	SA	4	3	0	3	0	0	0	0	1	0	0	0	0	1	0	0	0	0	1	0	0	3	0	0
SA-100	SA	55	2	0	2	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0
SA-116	SA	116	2	0	2	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	2	0	0
SA-125	SA	125	2	0	2	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	1	1	0
SA-13	SA	13	2	0	2	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	2	0	0
SA-17	SA	17	2	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	2	0	0
SA-20	SA	20	2	1	1	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0
SA-21	SA	21	2	0	2	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	1	1	0
SA-23	SA	23	2	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	2	0	0
SA-69	SA	69	2	0	2	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0
SA-90	SA	90	2	0	2	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0
SA-93	SA	93	2	1	1	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0
SA-99	SA	7	2	0	2	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	1	1	0	0
SA-10	SA	10	1	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
SA-120	SA	10	1	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
SA-24	SA	24	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0
SA-25	SA	25	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0
SA-30	SA	30	1	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
SA-39	SA	39	1	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
SA-43	SA	43	1	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0
SA-6	SA	6	1	0	0	0	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0
SA-8	SA	8	1	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
SA-83	SA	83	1	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
SA-9	SA	9	1	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
SA-94	SA	94	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0
SA-07	SA	7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
SA-101	SA	54	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
SA-102	SA	43	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
SA-103	SA	33	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

ID	Species	Faeces	Total	Fragment	Fibre	Filament	Sfere	Film	Ball	Blue	Green	Black	Purple	Transparent	White	Yellow	Red	Grey	Pink	Brown	Multicolour	Mesoplastic	LMP	SMPP	NP
SA-104	SA	32	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
SA-105	SA	30	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
SA-106	SA	23	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
SA-107	SA	20	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
SA-108	SA	108	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
SA-11	SA	11	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
SA-110	SA	110	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
SA-117	SA	117	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
SA-12	SA	12	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
SA-18	SA	18	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
SA-19	SA	19	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
SA-2	SA	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
SA-26	SA	26	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
SA-27	SA	27	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
SA-28	SA	28	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
SA-31	SA	31	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
SA-33	SA	33	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
SA-36	SA	36	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
SA-37	SA	37	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
SA-38	SA	38	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
SA-44	SA	44	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
SA-45	SA	45	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
SA-46	SA	46	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
SA-48	SA	48	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
SA-5	SA	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
SA-54	SA	54	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
SA-55	SA	55	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
SA-56	SA	56	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
SA-57	SA	57	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
SA-78	SA	78	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
SA-140	SA	84	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
SA-85	SA	85	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
SA-87	SA	87	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
SA-88	SA	88	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
SA-95	SA	95	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
SA-141	SA	98	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
SA-98	SA	98	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

ID	Species	Faeces	Total	Fragment	Fibre	Filament	Sfere	Film	Ball	Blue	Green	Black	Purple	Transparent	White	Yellow	Red	Grey	Pink	Brown	Multicolour	Mesoplastic	LMP	SMPP	NP
LM-5	LM	5	16	6	3	0	0	7	0	0	0	2	0	5	2	0	7	0	0	0	0	15	1	0	0
LM-46	LM	46	13	13	0	0	0	0	0	0	13	0	0	0	0	0	0	0	0	0	0	3	10	0	0
LM-13	LM	13	8	0	1	0	0	7	0	0	1	0	0	7	0	0	0	0	0	0	0	8	0	0	0
LM-8	LM	8	4	0	1	0	0	2	1	1	0	0	0	1	0	0	1	0	0	1	0	4	0	0	0
LM-32	LM	32	2	0	0	0	0	2	0	0	0	2	0	0	0	0	0	0	0	0	0	2	0	0	0
LM-34	LM	34	2	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	1	1	0	0
LM-43	LM	43	2	2	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	2	0	0	0
LM-44	LM	44	2	2	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	2	0	0	0
LM-6	LM	6	2	0	0	0	0	2	0	1	0	0	0	0	1	0	0	0	0	0	0	2	0	0	0
LM-18	LM	18	1	0	0	0	0	0	1	1	2	0	0	8	0	0	0	0	0	0	0	11	0	0	0
LM-29	LM	29	1	0	0	0	0	1	0	2	3	0	0	8	0	0	0	0	0	0	0	13	0	0	0
LM-38	LM	38	1	1	0	0	0	0	0	0	17	2	0	0	2	0	0	0	0	2	0	13	10	0	0
LM-74	LM	74	1	0	0	0	0	0	1	2	0	0	0	5	2	0	0	0	0	0	0	9	0	0	0
LM-9	LM	9	1	0	0	0	0	1	0	2	4	0	0	8	1	0	0	0	0	0	0	15	0	0	0
LM-100	LM	100	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
LM-101	LM	101	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
LM-102	LM	102	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
LM-103	LM	103	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
LM-104	LM	104	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
LM-105	LM	105	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
LM-106	LM	106	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
LM-107	LM	107	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
LM-108	LM	108	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
LM-109	LM	109	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
LM-110	LM	110	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
LM-111	LM	111	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
LM-112	LM	112	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
LM-113	LM	113	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
LM-114	LM	114	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
LM-115	LM	115	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
LM-116	LM	116	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
LM-117	LM	117	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
LM-118	LM	118	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
LM-119	LM	119	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
LM-120	LM	120	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
LM-121	LM	121	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
LM-122	LM	122	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
LM-123	LM	123	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
LM-124	LM	124	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
LM-125	LM	125	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
LM-126	LM	126	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
LM-127	LM	127	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
LM-128	LM	128	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
LM-129	LM	129	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
LM-130	LM	130	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
LM-131	LM	131	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
LM-132	LM	132	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
LM-133	LM	133	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
LM-134	LM	134	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
LM-135	LM	135	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
LM-136	LM	136	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
LM-137	LM	137	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
LM-75	LM	75	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
LM-76	LM	76	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
LM-77	LM	77	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
LM-78	LM	78	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
LM-79	LM	79	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
LM-80	LM	80	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
LM-81	LM	81	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
LM-82	LM	82	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
LM-83	LM	83	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
LM-84	LM	84	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
LM-85	LM	85	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
LM-86	LM	86	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
LM-87	LM	87	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
LM-88	LM	88	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
LM-89	LM	89	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
LM-90	LM	90	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
LM-91	LM	91	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
LM-92	LM	92	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
LM-93	LM	93	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
LM-94	LM	94	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
LM-95	LM	95	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
LM-96	LM	96	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
LM-97	LM	97	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
LM-98	LM	98	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
LM-99	LM	99	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

ID	Species	Faeces	Total	Fragment	Fibre	Filament	Sfere	Film	Ball	Blue	Green	Black	Purple	Transparent	White	Yellow	Red	Grey	Pink	Brown	Multicolour	Mesoplastic	LMP	SMPP	NP
IA-4	IA	4	3	0	0	0	0	3	0	0	0	0	0	2	1	0	0	0	0	0	0	3	0	0	0
IA-16	IA	16	1	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
IA-17	IA	17	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
IA-18	IA	18	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
IA-19	IA	19	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
IA-20	IA	20	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
IA-21	IA	21	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
IA-22	IA	22	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
IA-23	IA	23	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
IA-24	IA	24	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
IA-25	IA	25	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
IA-26	IA	26	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
IA-27	IA	27	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
IA-28	IA	28	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
IA-29	IA	29	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
IA-30	IA	30	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
IA-31	IA	31	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
IA-32	IA	32	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
IA-33	IA	33	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
IA-34	IA	34	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
IA-35	IA	35	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
IA-36	IA	36	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
IA-37	IA	37	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
IA-38	IA	38	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
IA-39	IA	39	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
IA-40	IA	40	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
IA-41	IA	41	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
IA-42	IA	42	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
IA-43	IA	43	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
IA-44	IA	44	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
IA-45	IA	45	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
IA-46	IA	46	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
IA-47	IA	47	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
IA-48	IA	48	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
IA-49	IA	49	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
IA-50	IA	50	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
IA-51	IA	51	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
IA-52	IA	52	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
IA-53	IA	53	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
IA-54	IA	54	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
IA-55	IA	55	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
IA-56	IA	56	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
IA-57	IA	57	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
IA-58	IA	58	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
IA-59	IA	59	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
IA-60	IA	60	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
IA-61	IA	61	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
IA-62	IA	62	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
IA-63	IA	63	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
IA-64	IA	64	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
IA-65	IA	65	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
IA-66	IA	66	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
IA-67	IA	67	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
IA-68	IA	68	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
IA-69	IA	69	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
IA-70	IA	70	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
IA-71	IA	71	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
IA-72	IA	72	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
IA-73	IA	73	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Table S5 –Sizes (mm) of each plastic retrieved from the faecal samples. Species are listed as follows: HH – Black-winged stilt, RA – Pied avocet, CA – Kentish plover, SA – Little tern, IA – Audouin’s gull, LM – Yellow-legged gull.

Mp ID	Size (mm)	Species
CA_10_01	5.483	CA
CA_11_01	2.65	CA
CA_11_02	0.822	CA
CA_13_01	1.962	CA
CA_13_02	0.434	CA
CA_13_03	0.256	CA
CA_14_01	0.611	CA
CA_17_01	7.698	CA
CA_17_02	1.108	CA
CA_17_03	1.152	CA
CA_2_01	1.302	CA
CA_2_02	0.47	CA
CA_22_01	1.139	CA
CA_24_01	0.864	CA
CA_3_01	3.442	CA
CA_3_02	1.146	CA
CA_30_01	0.537	CA
CA_30_02	2.697	CA
CA_34_01	4.2	CA
CA_34_02	2.061	CA
CA_35_01	3.725	CA
CA_35_02	3.896	CA
CA_35_03	1.139	CA
CA_35_04	0.239	CA
CA_36_01	0.935	CA
CA_38_1	0.45	CA
CA_4_01	3.825	CA
CA_5_01	1.435	CA
CA_5_02	0.336	CA
CA_5_03	1.447	CA
CA_5_04	1.531	CA
CA_5_05	1.442	CA
CA_5_06	0.775	CA
CA_5_07	1.542	CA
CA_6_01	3.757	CA
CA_8_01	0.699	CA
CA_8_02	0.4	CA
CA_9_01	0.15	CA
CA_9_02	0.191	CA
HH_39_01	0.726	HH
HH_39_02	1.265	HH
HH_39_03	2.053	HH
HH_41_01	0.754	HH
HH_42_01	1.084	HH
HH_42_02	0.642	HH
HH_42_03	1.437	HH
HH_43_01	1.606	HH
HH_45_01	7.854	HH
HH_45_02	3.354	HH
HH_45_03	1.606	HH
HH_46_01	1.789	HH
HH_46_02	1.248	HH
HH_46_03	4.521	HH
HH_46_04	2.35	HH
HH_46_05	0.487	HH
HH_46_06	0.965	HH
HH_46_07	4.703	HH
IA_47_01	1.293	IA
IA_47_02	0.173	IA
IA_48_01	1.555	IA
IA_49_01	4.047	IA
IA_49_02	1.165	IA
IA_49_03	0.26	IA
IA_53_01	1.936	IA
IA_53_02	2.077	IA
IA_54-1_01	3.054	IA
IA_57_01	2.501	IA
IA_58_01	3.489	IA
IA_58_02	2.175	IA
IA_58_03	2.745	IA
IA_60_01	0.394	IA
IA_62_01	0.894	IA
IA_63_01	1.405	IA
IA_64_01	2.032	IA
IA_64_02	0.137	IA
IA_64_03	0.625	IA
IA_66_01	1.293	IA
IA_67_01	0.445	IA
IA_67_02	0.843	IA
IA_67_03	0.839	IA
IA_70_01	1.254	IA
IA_71_01	1.293	IA
IA_71_02	1.72	IA
IA_71_03	0.468	IA
IA_71_04	2.424	IA
IA_71_05	3.231	IA
IA_71_06	2.05	IA
IA_72_01	4.226	IA
IA_72_02	0.16	IA
IA_73_01	2.987	IA
IA_73_02	5.096	IA
IA_73_03	1.75	IA
IA_76_01	3.25	IA
IA_76_02	1.109	IA
IA_77_01	1.773	IA
IA_78_01	0.978	IA
LM_102_02	6.41	LM
LM_102_03	5.24	LM
LM_102_04	0.942	LM
LM_102_06	2.024	LM
LM_102_07	0.927	LM
LM_102_08	0.927	LM
LM_105_01	2.192	LM
LM_105_02	0.679	LM
LM_105_03	3.729	LM
LM_105_04	1.826	LM
LM_105_06	13.03	LM
LM_105_07	2.428	LM
LM_105_08	0.927	LM
LM_105_09	0.927	LM
LM_105_10	1.003	LM
LM_105_11	0.927	LM
LM_106_01	1.276	LM
LM_107_01	3.859	LM
LM_107_02	1.777	LM
LM_107_03	1.473	LM
LM_107_04	3.31	LM
LM_107_05	2.897	LM
LM_107_06	1.531	LM
LM_107_07	1.611	LM
LM_108_01	4.555	LM
LM_108_02	2.993	LM
LM_108_03	7.011	LM
LM_108_04	2.207	LM
LM_108_05	9.126	LM
LM_108_06	1.968	LM
LM_108_07	0.695	LM
LM_108_08	3.412	LM
LM_108_09	2.27	LM
LM_108_10	1.264	LM
LM_108_10	0.809	LM
LM_108_10	1.352	LM
LM_108_11	1.292	LM
LM_108_11	0.515	LM
LM_109_01	1.18	LM
LM_109_02	3.017	LM
LM_109_03	1.199	LM
LM_110_01	0.635	LM
LM_110_02	3.527	LM
LM_110_03	0.831	LM
LM_110_04	1.72	LM
LM_80_01	1.96	LM
LM_80_02	1.675	LM
LM_81_01	1.931	LM
LM_81_02	2.512	LM
LM_81_02	2.712	LM
LM_81_02	0.563	LM
LM_81_02	0.719	LM
LM_81_03	0.927	LM
LM_81_04	2.247	LM
LM_81_04	1.257	LM
LM_81_04	0.846	LM
LM_81_04	1.861	LM
LM_81_05	0.927	LM
LM_81_06	1.196	LM
LM_81_07	0.114	LM
LM_81_08	2.227	LM
LM_81_09	0.686	LM
LM_82_02	1.428	LM
LM_83_01	0.167	LM
LM_83_02	0.172	LM
LM_84_01	0.532	LM
LM_85_03	1.127	LM
LM_85_04	0.437	LM
LM_85_05	4.321	LM
LM_85_06	1.885	LM
LM_85_07	4.138	LM
LM_86_02	0.665	LM
LM_86_03	0.076	LM
LM_86_03	0.057	LM
LM_86_03	0.063	LM
LM_86_03	0.047	LM
LM_86_04	0.052	LM
LM_88_01	2.319	LM
LM_88_02	1.356	LM
LM_88_03	1.722	LM
LM_88_04	3.712	LM
LM_88_05	1.382	LM
LM_89_02	7.352	LM
LM_89_03	0.721	LM
LM_89_04	2.451	LM
LM_89_07	2.813	LM
LM_91_02	1.241	LM
LM_91_04	0.927	LM
LM_91_05	0.71	LM
LM_91_06	1.625	LM
LM_91_07	6.515	LM
LM_91_07	3.371	LM
LM_91_08	2.359	LM
LM_91_09	0.153	LM
LM_91_10	3.92	LM
LM_91_11	8.821	LM
LM_91_12	1.897	LM
LM_91_13	1.037	LM
LM_91_14	2.824	LM
LM_91_15	8.88	LM
LM_91_17	4.924	LM
LM_94_02	2.351	LM
LM_94_03	2.892	LM
LM_94_04	1.953	LM
LM_96_01	0.721	LM
LM_96_02	3.246	LM
LM_96_04	1.565	LM
LM_96_04	3.121	LM
LM_96_06	0.639	LM
LM_96_07	2.783	LM
LM_96_08	3.135	LM
LM_96_09	3.183	LM
LM_96_11	0.676	LM
LM_97_02	1.47	LM
LM_97_03	1.685	LM
LM_97_04	2.506	LM
LM_98_02	1.979	LM
LM_98_03	2.339	LM
LM_98_04	3.302	LM
LM_99_01	1.074	LM
LM_99_02	0.4	LM
LM_99_03	3.344	LM
LM_99_04	0.115	LM
LM_99_06	0.071	LM
LM_99_07	0.927	LM
LM_99_08	0.927	LM
LM_99_09	0.574	LM
LM_99_10	0.195	LM
LM_99_11	0.927	LM
LM_99_12	0.266	LM
LM_99_13	0.927	LM
LM_99_14	0.927	LM
RA_113_01	0.851	RA
RA_113_03	1.062	RA
RA_115_02	1.879	RA
RA_115_03	0.885	RA
RA_118_01	1.111	RA
RA_118_02	0.645	RA
RA_118_03	3.115	RA
RA_120_01	0.376	RA
RA_120_02	0.756	RA
RA_120_04	0.866	RA
RA_120_06	2.321	RA
RA_120_07	1.897	RA
RA_121_01	2.189	RA
RA_121_02	1.236	RA
RA_121_03	1.377	RA
RA_121_04	1.72	RA
RA_121_05	1.409	RA
RA_121_06	0.818	RA
RA_121_07	1.07	RA
RA_121_08	0.924	RA
RA_121_09	1.192	RA
RA_121_10	1.346	RA
RA_121_11	0.669	RA
RA_121_12	1.062	RA
RA_121_13	0.779	RA
RA_121_14	1.062	RA
RA_125_01	4.277	RA
RA_125_02	1.149	RA
RA_126_01	1.062	RA
RA_128_01	1.87	RA
RA_128_02	2.275	RA
RA_128_03	3.25	RA
RA_128_05	0.786	RA
RA_128_06	3.629	RA
RA_128_07	1.062	RA
RA_128_12	2.265	RA
RA_129_01	0.841	RA
RA_129_02	1.197	RA
RA_129_04	1.14	RA
RA_130_01	1.391	RA
RA_131_01	0.919	RA
RA_131_02	0.613	RA
RA_131_03	0.219	RA
RA_133_01	1.606	RA
RA_134_03	1.043	RA
RA_134_04	8.397	RA
RA_135_01	0.744	RA
RA_136_01	2.205	RA
RA_136_02	1.172	RA
RA_136_03	0.264	RA
RA_136_04	4.129	RA
RA_136_05	0.163	RA
RA_136_06	1.104	RA
RA_137_02	3.237	RA
RA_138_01	1.062	RA
RA_138_02	0.173	RA
RA_139_01	0.478	RA
RA_139_02	0.405	RA
RA_139_03	0.159	RA
RA_140_01	0.696	RA
RA_140_02	0.861	RA
RA_142_03	1.062	RA
SA_144_01	0.603	SA
SA_145_01	2.491	SA
SA_146_01	1.289	SA
SA_146-2_02	1.267	SA
SA_148_01	0.438	SA
SA_150_01	1.426	SA
SA_150_02	0.775	SA
SA_150_03	4.56	SA
SA_150_03	0.607	SA
SA_154_01	0.422	SA
SA_154_02	2.836	SA
SA_158_E16_01	0.208	SA
SA_160_B17_01	1.159	SA
SA_161_C17_01	0.157	SA
SA_164_A18_01	0.261	SA
SA_166-2_C18_05	2.862	SA
SA_172_D19_03	0.472	SA

Table S6 – Sizes (mm) of each plastic retrieved from the regurgitates. Please note that in the case of sample 84, which had 166 similar plastics, a mean size value was calculated based in the measurements of 3 of those plastics. Species presented are listed as follows: HH – Black-winged stilt, RA – Pied avocet, CA – Kentish plover, SA – Little tern, IA – Audouin's gull, LM – Yellow-legged gull.

Mp ID	Size (mm)	Species
LM-1	4	LM
LM-2	15	LM
LM-3	5	LM
LM-4	15	LM
LM-5	21	LM
LM-6	35	LM
LM-7	25	LM
LM-8	23	LM
LM-9	9	LM
LM-10	7	LM
LM-11	8	LM
LM-12	7	LM
LM-13	8	LM
LM-14	12	LM
LM-15	16	LM
LM-16	5	LM
LM-17	30	LM
LM-18	36	LM
LM-19	11	LM
LM-20	43	LM
LM-21	26	LM
LM-22	14	LM
LM-23	74	LM
LM-24	17	LM
LM-25	71	LM
LM-26	26	LM
LM-27	17	LM
LM-28	19	LM
LM-29	18	LM
LM-30	11	LM
LM-31	12	LM
LM-32	13	LM
LM-33	11	LM
LM-34	28	LM
LM-35	67	LM
LM-36	7	LM
LM-37	1	LM
LM-38	5	LM
LM-39	17	LM
LM-40	9	LM
LM-41	18	LM
LM-42	7	LM
LM-43	8	LM
LM-44	6	LM
LM-45	4	LM
LM-46	4	LM
LM-47	3	LM
LM-48	5	LM
LM-49	4	LM
LM-50	3	LM
LM-51	3	LM
LM-52	2	LM
LM-53	2	LM
LM-54	3	LM
LM-55	3	LM
LM-56	6	LM
IA-1	11	IA
IA-2	5	IA
IA-3	19	IA
IA-4	19	IA
8T_1_S	2.98	SA
20N_1_S	0.673	SA
20N_2_S	1.234	SA
6O_1_S	1.945	SA
21O_1_S	2.88	SA
21O_2_S	0.615	SA
24T_1_S	2.606	SA
24T_1_S	1.526	SA
4O_1_S	1.016	SA
4O_2_S	1.666	SA
4O_3_S	1.318	SA
13O_1_S	2.412	SA
13O_2_S	1.388	SA
9T_1_S	0.356	SA
1N_1_S	1.089	SA
1N_2_S	1.637	SA
1N_3_S	0.536	SA
1N_4_S	1.751	SA
25A_1_S	3.752	SA
34O_1_S	0.635	SA
34O_2_S	1.184	SA
34O_3_S	2.514	SA
84-5	0.6525	SA
84-6	0.6525	SA
84-7	0.6525	SA
84-8	0.6525	SA
84-9	0.6525	SA
84-10	0.6525	SA
84-11	0.6525	SA
84-12	0.6525	SA
84-13	0.6525	SA
84-14	0.6525	SA
84-15	0.6525	SA
84-16	0.6525	SA
84-17	0.6525	SA
84-18	0.6525	SA
84-19	0.6525	SA
84-20	0.6525	SA
84-21	0.6525	SA
84-22	0.6525	SA
84-23	0.6525	SA
84-24	0.6525	SA
84-25	0.6525	SA
84-26	0.6525	SA
84-27	0.6525	SA
84-28	0.6525	SA
84-29	0.6525	SA
84-30	0.6525	SA
84-31	0.6525	SA
84-32	0.6525	SA
84-33	0.6525	SA
84-34	0.6525	SA
84-35	0.6525	SA
84-36	0.6525	SA
84-37	0.6525	SA
84-38	0.6525	SA
84-39	0.6525	SA
84-40	0.6525	SA
84-41	0.6525	SA
84-42	0.6525	SA
84-43	0.6525	SA
84-44	0.6525	SA
84-45	0.6525	SA
84-46	0.6525	SA
84-47	0.6525	SA
84-48	0.6525	SA
84-49	0.6525	SA
84-50	0.6525	SA
84-51	0.6525	SA
84-52	0.6525	SA
84-53	0.6525	SA
84-54	0.6525	SA
84-55	0.6525	SA
84-56	0.6525	SA
84-57	0.6525	SA
84-58	0.6525	SA
84-59	0.6525	SA
84-60	0.6525	SA
84-61	0.6525	SA
84-62	0.6525	SA
84-63	0.6525	SA
84-64	0.6525	SA
84-65	0.6525	SA
84-66	0.6525	SA
84-67	0.6525	SA
84-68	0.6525	SA
84-69	0.6525	SA
84-70	0.6525	SA
84-71	0.6525	SA
84-72	0.6525	SA
84-73	0.6525	SA
84-74	0.6525	SA
84-75	0.6525	SA
84-76	0.6525	SA
84-77	0.6525	SA
84-78	0.6525	SA
84-79	0.6525	SA
84-80	0.6525	SA
84-81	0.6525	SA
84-82	0.6525	SA
84-83	0.6525	SA
84-84	0.6525	SA
84-85	0.6525	SA
84-86	0.6525	SA
84-87	0.6525	SA
84-88	0.6525	SA
84-89	0.6525	SA
84-90	0.6525	SA
84-91	0.6525	SA
84-92	0.6525	SA
84-93	0.6525	SA
84-94	0.6525	SA
84-95	0.6525	SA
84-96	0.6525	SA
84-97	0.6525	SA
84-98	0.6525	SA
84-99	0.6525	SA
84-100	0.6525	SA
84-101	0.6525	SA
84-102	0.6525	SA
84-103	0.6525	SA
84-104	0.6525	SA
84-105	0.6525	SA
84-106	0.6525	SA
84-107	0.6525	SA
84-108	0.6525	SA
84-109	0.6525	SA
84-110	0.6525	SA
84-111	0.6525	SA
84-112	0.6525	SA
84-113	0.6525	SA
84-114	0.6525	SA
84-115	0.6525	SA
84-116	0.6525	SA
84-117	0.6525	SA
84-118	0.6525	SA
84-119	0.6525	SA
84-120	0.6525	SA
84-121	0.6525	SA
84-122	0.6525	SA
84-123	0.6525	SA
84-124	0.6525	SA
84-125	0.6525	SA
84-126	0.6525	SA
84-127	0.6525	SA
84-128	0.6525	SA
84-129	0.6525	SA
84-130	0.6525	SA
84-131	0.6525	SA
84-132	0.6525	SA
84-133	0.6525	SA
84-134	0.6525	SA
84-135	0.6525	SA
84-136	0.6525	SA
84-137	0.6525	SA
84-138	0.6525	SA
84-139	0.6525	SA
84-140	0.6525	SA
84-141	0.6525	SA
84-143	0.6525	SA
84-144	0.6525	SA
84-145	0.6525	SA
84-146	0.6525	SA
84-147	0.6525	SA
84-148	0.6525	SA
84-149	0.6525	SA
84-150	0.6525	SA
84-151	0.6525	SA
84-152	0.6525	SA
84-153	0.6525	SA
84-154	0.6525	SA
84-155	0.6525	SA
84-156	0.6525	SA
84-157	0.6525	SA
84-158	0.6525	SA
84-159	0.6525	SA
84-160	0.6525	SA
84-161	0.6525	SA
84-162	0.6525	SA
84-163	0.6525	SA
84-164	0.6525	SA
84-165	0.6525	SA
84-166	0.6525	SA
84-167	0.6525	SA
84-168	0.6525	SA
32-26	0.153	SA
32-27	0.153	SA
32-28	0.153	SA
32-29	0.153	SA
32-30	0.153	SA
32-31	0.153	SA
32-32	0.153	SA
96-3	0.354	SA
96-4	0.354	SA
96-5	0.354	SA
96-6	0.354	SA
96-7	0.354	SA
96-8	0.354	SA
96-9	0.354	SA
96-10	0.354	SA
96-11	0.354	SA
96-12	0.354	SA
96-13	0.354	SA
96-14	0.354	SA
96-15	0.354	SA
96-16	0.354	SA
96-17	0.354	SA
96-18	0.354	SA
96-19	0.354	SA
96-20	0.354	SA
96-21	0.354	SA
96-22	0.354	SA
96-23	0.354	SA
96-24	0.354	SA
96-25	0.354	SA
96-26	0.354	SA
96-27	0.354	SA
96-28	0.354	SA
96-29	0.354	SA
96-30	0.354	SA
96-31	0.354	SA
96-32	0.354	SA
96-33	0.354	SA
96-34	0.354	SA
89-2	0.142	SA
89-3	0.142	SA
89-4	0.142	SA
89-5	0.142	SA
89-6	0.142	SA
89-7	0.142	SA
89-8	0.142	SA
89-9	0.142	SA
89-10	0.142	SA
89-11	0.142	SA
89-12	0.142	SA
89-13	0.142	SA
89-14	0.142	SA
89-15	0.142	SA
89-16	0.142	SA
89-17	0.142	SA
89-18	0.142	SA
84S_1_S	0.637	SA
84S_2_S	0.668	SA
84S_3_S	1.034	SA
10T_1_S	1.234	SA
39T_1_S	0.285	SA
90C_1_P	0.947	SA
90C_2_P	1.714	SA
17F_1_P	1.453	SA
17F_2_P	2.194	SA
43F_1_P	0.275	SA
30F_1_P	1.53	SA
94C_1_P	2.183	SA
24F_1_P	2.819	SA
128D_1_P	0.579	SA
128D_2_P	1.997	SA
128D_3_P	1.553	SA
69F_1_P	0.821	SA
69F_2_P	0.747	SA
32F_1_P	0.153	SA
32F_2_P	2.779	SA
93C_1_P	4.114	SA
93C_2_P	0.326	SA
10F_1_P	2.02	SA
83C_1_P	2.406	SA
7F_1_P	1.054	SA
7F_2_P	23.986	SA
55-2_1_P	1.776	SA
55-2_2_P	1.493	SA
96C_1_P	0.354	SA
125D_1_P	0.822	SA
125D_2_P	4.39	SA
89C_1_P	0.142	SA
116C_1_P	1.246	SA
116C_2_P	1.275	SA

Table S7 – Dry weight (g) of each sample and total number of plastics found in faecal samples. Only samples with at least one plastic are presented. Species are listed as follows: HH – Black-winged stilt, RA – Pied avocet, CA – Kentish plover, SA – Little tern, IA – Audouin's gull, LM – Yellow-legged gull.

Species	Faeces	N.microplasitcs	Dry weight (g)
SA	172	1	0.260675
SA	166	1	0.293075
SA	164	1	0.297375
SA	161	1	0.321675
SA	160	1	0.346175
SA	158	1	0.357075
SA	154	2	0.400475
SA	150	4	0.507075
SA	148	1	0.678375
SA	146	2	0.844575
SA	145	1	0.859275
SA	144	1	1.013675
RA	142	1	0.460175
RA	140	2	0.491875
RA	139	3	0.537075
RA	138	2	0.613475
RA	137	1	0.674575
RA	136	6	0.698875
RA	135	1	0.742175
RA	134	2	0.753275
RA	133	1	0.780275
RA	131	3	0.817775
RA	130	1	0.861375
RA	129	3	1.017275
RA	128	7	1.039975
RA	126	1	1.107675
RA	125	2	1.121275
RA	121	14	1.415375
RA	120	5	1.436575
RA	118	3	1.591975
RA	115	2	1.663175
RA	113	2	2.095675
LM	110	4	0.930375
LM	109	3	1.035575
LM	108	14	1.194575
LM	107	7	1.251875
LM	106	1	1.317275
LM	105	10	1.346075
LM	102	6	1.459375
LM	99	14	1.629075
LM	98	3	1.629375
LM	97	3	1.696375
LM	96	9	1.735575
LM	94	3	1.796775
LM	91	15	1.868575
LM	89	4	2.061475
LM	88	5	2.140175
LM	86	6	2.232175
LM	85	5	2.266275
LM	84	1	2.274075
LM	83	2	2.429375
LM	82	1	2.503975
LM	81	15	2.685575
LM	80	2	2.805375
IA	78	1	0.389475
IA	77	1	0.473575
IA	76	2	0.491175
IA	73	3	0.819775
IA	72	2	0.887675
IA	71	6	0.942475
IA	70	1	1.188075
IA	67	3	1.246075
IA	66	1	1.250975
IA	64	3	1.366875
IA	63	1	1.407175
IA	62	1	1.509575
IA	60	1	1.581075
IA	58	3	1.607475
IA	57	1	1.617275
IA	54	1	1.683475
IA	53	2	1.704275
IA	49	3	1.825075
IA	48	1	1.835675
IA	47	2	1.870075
HH	46	7	0.163875
HH	45	3	0.228675
HH	43	1	0.324275
HH	42	3	0.431675
HH	41	1	0.463675
HH	39	3	0.600575
CA	38	1	0.9356
CA	36	1	0.9702
CA	35	4	0.9775
CA	34	2	0.9793
CA	30	2	0.9949
CA	24	1	1.0191
CA	22	1	1.0268
CA	17	3	1.0422
CA	14	1	1.0531
CA	13	3	1.055
CA	11	1	1.0597
CA	10	1	1.0789
CA	9	2	1.091
CA	8	2	1.0921
CA	6	1	1.1102
CA	5	7	1.1423
CA	4	1	1.1521
CA	3	2	1.1548
CA	2	2	1.2754

Table S8 – Concentrations of PBDE's found in each bird species egg sample. Values presented in ng/500mg of egg sample. Species presented are listed as follows: HH – Black-winged stilt, RA – Pied avocet, CA – Kentish plover, SA – Little tern, IA – Audouin's gull, LM – Yellow-legged gull.

Species	BDE28	BDE47	BDE99	BDE154	BDE153	BDE183
LM	86.2126103219487	21.0487588177251	8.80438526096915	1.11988255341027	3.79548795242797	1.46018338565481
LM	84.3373756340637	6.48755227243233	2.98694254062428	0.721589499073436	2.81900454049843	1.03623531298146
LM	84.1631877871247	5.33728914033547	2.23899404261553	0.481556457630577	2.01050640836263	0.91157963547394
LM	83.7021591374173	2.355474830066	1.51045991527513	0.333710436610675	1.91555379604402	0.855162774318231
LM	82.2509884962687	2.20634713965547	0.432110202198368	0.332661648275324	1.8453977512379	0.564803006787272
LM	77.5526071929491	1.51157334132112	0.0283699002827924	0.301685040093597	1.80300500106005	0.282103377063645
LM	76.4841566125363	0.89175023290006	0.0235153635587947	0.283011841801437	1.77649698675084	0
LM	69.92475097163	0.622523545388496	0	0.279566392160755	1.76762033849129	0
LM	0	0.52613716010211	0	0.264224833390381	1.737130748605	0
LM	0	0.294176869090571	0	0.219735851051396	0	0
HH	79.2207398751849	0.762774548044651	0.807450683791297	0.261001413817837	1.76654809977245	0
HH	82.6974904179695	0.207547733345434	0	0.363352744685178	1.70171235001091	0.974917811391898
HH	22.0872859337228	17.1523677925806	12.4716899518018	0.253615832372932	2.04595084205944	1.08621392392745
HH	42.3884818484287	10.6757003816716	8.91512076538813	0.407586867465458	2.07165179126056	1.29540871700864
HH	74.7854964904988	3.77740557953978	2.54755273989807	0.278269472264783	0	0.73731888701947
HH	78.8515742318986	14.3242489634232	12.2082603905773	0.280622260957	1.75165816663557	0.389608161162569
HH	0	0.299599302726289	0	0.249360325940389	1.88116605465358	0.67738861608088
HH	82.8542547175031	0.50777679909374	0	0.226356152980437	1.71813168478827	0
HH	21.3704993629395	17.9057924392422	5.50510812833802	0.273842120235018	2.42239049120025	0.760930658487655
HH	81.5879391720792	2.32376672195102	0.967553260821642	0.29596710382646	1.70514868952827	0.715917170698234
RA	86.3715585103641	5.63132102255566	0.522538659055919	0.47988065506322	1.93766780984818	1.06431546408042
RA	85.7050310768313	4.23686121539732	0.50828590211241	0.358908098044387	1.88443274763263	1.03945727302071
RA	81.9010090478938	1.74963556219963	0.221503564637249	0.341793487710597	1.85337879752942	0.912182663286841
RA	67.7015441813325	1.48407731596726	0.196640221544146	0.334270287632822	1.84510196100569	0.821547206549718
RA	55.3566390129524	0.958611483328896	0.194781045985769	0.329165541739869	1.80275886867063	0.67807086320906
RA	41.3222991328565	0.882915097642811	0	0.319670061424653	1.78617148337074	0.567948015298952
RA	41.0050688519825	0.747258528102318	0	0.301205646390754	1.7614995852848	0
RA	0	0.565913066906303	0	0.256923209770108	1.73928598654814	0
RA	0	0.509075532351217	0	0.25190280004759	1.71554852013123	0
RA	0	0.351514801169784	0	0.203297252311435	0	0
SA	75.1184384735063	6.01254596376425	1.37150090736353	0.259887873944422	2.05486665572557	0.364296399460953
SA	84.715195882212	0.899433506432763	0	0.492939738495148	1.70900607014978	0.541582746896012
SA	84.3053241874537	0.227194939600549	0	0.212025482660648	1.76946751594484	0.798843896928027
SA	80.9765066574908	0.513216931959276	0	0.465295614830529	1.71015873811557	0.769535065989713
SA	39.1532780521997	1.58798687005693	0.12465413019299	0.112199938526994	1.97407035673674	0.273796500035781
SA	75.280312458554	0.80771175653799	0.0109221136764497	0.390123607081902	1.75738267737454	0.866494643499566
SA	82.9434177780904	0.637526589364529	0	0	1.71960725332682	1.19558946427993
SA	38.9506577877165	1.91160850501811	0.50974521725446	0.259312733923379	1.85180508919129	0.618426598795788
SA	0	19.6892076830762	1.41910121735764	0.2252823861726	1.6894812251809	0
SA	75.6106165711172	0.669676016588593	0.233311370610199	0.322010078973794	1.7705464772295	0.606532930234569
CA	0	1.41117702445051	0.527727809312423	0.487224416017594	1.74242137978319	0.632567788526007
CA	80.2382741938319	0.62028369180127	0.146174443755962	0.345132674778696	1.87565309103284	1.13074963790153
CA	85.2098929034201	0.417778445374806	0	0.295027587763207	1.69985494150799	0.590234218394469
CA	82.596308966429	1.84504250010561	1.21216361397878	0	1.93930402549124	0
CA	80.8986848092333	0.363958176707536	0	0.226184065120402	1.716193189199	0.559871126037058
CA	83.4703747714843	0.722328212635829	0.142972739550088	0.231061678908073	1.77762337322646	1.00384521509353
CA	0	8.83354649018385	5.80680633887195	0.564578675588424	1.86317405408041	0.67222011277059
CA	0	6.9591292585332	5.87694749474369	1.69136546130911	1.86529476451617	1.03296537525577
CA	0	1.06106254479317	0.906307979282025	0.265901419941584	0.94558036157142	0.890522568677503
CA	85.7959322495204	0.36777905388513	0	0.267229254908056	1.70156680516129	1.65829083767733
IA	85.4124834342899	20.0343780888215	15.3638847882948	0.679156775323451	2.21171348639421	1.56205699875598
IA	85.3963417555744	3.09544217293958	2.65547634043357	0.607792020405194	2.09943330171722	1.32993236411569
IA	85.0966426250065	2.16330944497161	1.29784286896141	0.571895871532437	1.91721047588542	1.24340322695345
IA	84.4107147423105	1.74699403497627	1.18497989509322	0.297449483407086	1.89811501081006	0.930174424349608
IA	83.78421087748	1.65245500411182	1.17158955772732	0.280533351566952	1.80804384695066	0.606119851359939
IA	81.1208585215774	1.33705826589169	1.0312590876736	0.259220065478496	1.77435497872182	0.543432150322094
IA	80.502718978969	0.957492867580896	0.88991987618178	0.239912125348192	1.70560931321192	0.514958265786755
IA	0	0.91886707322615	0.72653176631492	0.239057894332293	1.70529647604854	0.503548836203065
IA	0	0.339711807590641	0	0.225077039821769	0.943291878452975	0.372015200661772
IA	0	0.159220580370093	0	0	0.88685160374454	0

Table S9 – Generalized Linear models used to assess the effect of bird species (HH – Black-winged stilt, RA – Pied avocet, CA – Kentish plover, SA – Little tern, IA – Audouin's gull, LM – Yellow-legged gull) on the presence and number of plastics and their shapes (Fibre, Fragments) in faeces. Each model used is identified on the left. Associated ß±SE, Z and p values are presented. Highlighted in red are significant values (<0.05). At the bottom, zero inflation tests (ratio) and performance of the models performed on quantitative data are shown. Highlighted in red are the models selected in each case.

		Faeces																			
		Total$Species							Fibre$Species							Fragments$Species					
		CA	HH	IA	LM	RA	SA		CA	HH	IA	LM	RA	SA		CA	HH	IA	LM	RA	SA
Binomial - PRESENCE	Presence																				
	ß	0.05407	1.04455	0.45676	0.73439	0.37672	-0.51360	ß	-0.05407	1.15268	0.43356	0.70069	0.23639	-0.40547	ß	-2.1102	-17.4559	0.6439	0.4238	0.3874	-17.4559
	SE (±)	0.32892	0.88026	0.49145	0.50363	0.48488	0.49412	SE (±)	0.32892	0.88026	0.48759	0.49670	0.48001	0.49412	SE (±)	0.5294	3802.1178	0.6967	0.7193	0.7183	1931.4778
	Z	0.164	1.187	0.929	1.458	0.777	-1.039	Z	-0.164	1.309	0.889	1.411	0.492	-0.821	Z	-3.986	-0.005	0.924	0.589	0.539	-0.009
	P	0.869	0.235	0.353	0.145	0.437	0.299	P	0.869	0.190	0.374	0.158	0.622	0.412	P	6.73e-05	0.996	0.355	0.556	0.590	0.993
Poisson																					
	ß	0.02667	0.78426	0.17116	1.39794	0.60396	-0.62744	ß	-0.1452	0.9561	0.2058	1.3979	0.6560	-0.4556	ß	-2.0015	-16.3011	0.1452	1.5315	0.4509	-16.3011
	SE (±)	0.16221	0.28613	0.22793	0.18393	0.20601	0.29176	SE (±)	0.1768	0.2946	0.2463	0.2004	0.2223	0.3001	SE (±)	0.4472	2021.4529	0.6325	0.5000	0.5855	1026.8992
	Z	0.164	2.741	0.751	7.600	2.932	-2.151	Z	-0.821	3.245	0.836	6.974	2.951	-1.518	Z	-4.475	-0.008	0.230	3.063	0.770	-0.016
	P	0.86941	0.00613	0.45270	2.95e-14	0.00337	0.03151	P	0.41149	0.00117	0.40338	3.08e-12	0.00317	0.12901	P	7.63e-06	0.99357	0.81844	0.00219	0.44128	0.98733
Zero Inflated																					
	ß	0.46601	0.57123	-0.04329	1.33089	0.61081	-0.76399	ß	0.1415	0.8958	0.1236	1.5275	0.9860	-0.4395	ß	-7.675e-01	-3.828e-05	-1.089e+00	2.134e+00	4.326e-01	-1.483e-04
	SE (±)	0.21055	0.33461	0.29806	0.22795	0.25227	0.47430	SE (±)	0.2585	0.3667	0.3522	0.2756	0.2969	0.4974	SE (±)	9.663e-01	NaN	1.065e+00	9.940e-01	1.178e+00	NaN
	Z	2.213	1.707	-0.145	5.839	2.421	-1.611	Z	0.547	2.443	0.351	5.542	3.321	-0.883	Z	-0.794	NaN	-1.022	2.147	0.367	NaN
	P	0.0269	0.0878	0.8845	5.27e-09	0.0155	0.1072	P	0.584103	0.014564	0.725721	2.99e-08	0.000897	0.376993	P	0.4271	NaN	0.3066	0.0318	0.7134	NaN
Zero inflated w/ negative binomial dist																					
	ß	0.13624	0.67473	0.06159	1.55684	0.71550	-0.73701	ß	-0.1452	0.9561	0.2058	1.6795	1.0393	-0.4556	ß	-1.444e+00	-4.686e-05	-4.128e-01	2.506e+00	4.718e-01	-1.816e-04
	SE (±)	0.31499	0.48812	0.38174	0.36394	0.38619	0.42378	SE (±)	0.2253	0.4432	0.3204	0.3158	0.3555	0.3645	SE (±)	1.248e+00	NaN	1.339e+00	1.264e+00	1.410e+00	NaN
	Z	0.433	1.382	0.161	4.278	1.853	-1.739	Z	-0.644	2.157	0.642	5.318	2.923	-1.250	Z	-1.157	NaN	-0.308	1.983	0.335	NaN
	P	0.6654	0.1669	0.8718	1.89e-05	0.0639	0.0820	P	0.51929	0.03097	0.52072	1.05e-07	0.00346	0.21130	P	0.2474	NaN	0.7578	0.0473	0.7379	NaN
Zero inflation																					
	Observed zeros	74						Observed zeros	79						Observed zeros	154					
	Predicted zeros	47						Predicted zeros	53						Predicted zeros	143					
	Ratio	0.64						Ratio	0.67						Ratio	0.93					
	Prob 0 inflation	Yes						Prob 0 inflation	Yes						Prob 0 inflation	Yes					
Performance		Zero inflated	Poisson			Negative binomial distribution			Zero inflated	Poisson			Negative binomial distribution			Zero inflated	Poisson			Negative binomial distribution	
	AIC	649.733	728.402			601.919		AIC	616.299	691.944			569.047		AIC	177.604	216.022			172.325	
	Log scores	-1.808	-2.071			0.064		Log scores	-1.712	-1.965			0.065		Log scores	0.072	-0.590			0.072	

Table S10 – Generalized Linear models used to assess the effect of bird species (HH – Black-winged stilt, RA – Pied avocet, CA – Kentish plover, SA – Little tern, IA – Audouin's gull, LM – Yellow-legged gull) on presence and number of plastics and their colours (Blue, Transparent) in faeces. Each model used is identified on the left. Associated ß±SE, Z and p values are presented. Highlighted in red are significant values (<0.05). At the bottom, zero inflation tests (ratio) and performance of the models performed on quantitative data are shown. Highlighted in red are the models selected in each case.

		Faeces - Colours												
		Blue$Species							Transparent$Species					
		CA	HH	IA	LM	RA	SA		CA	HH	IA	LM	RA	SA
Binomial - PRESENCE	Presence													
	ß	-0.38299	0.38299	-0.12783	0.38299	0.07761	-1.04412	ß	-2.1102	0.1643	1.1719	2.2354	1.2773	-0.5639
	SE (±)	0.33485	0.78238	0.49543	0.48695	0.48598	0.56461	SE (±)	0.5294	1.1930	0.6595	0.6370	0.6510	0.9027
	Z	-1.144	0.490	-0.258	0.787	0.160	-1.849	Z	-3.986	0.138	1.777	3.509	1.962	-0.625
	P	0.2527	0.6245	0.7964	0.4316	0.8731	0.0644	P	6.73e-05	0.89046	0.07555	0.00045	0.04975	0.53213
Poisson														
	ß	-0.3151	0.8747	-0.5116	0.4047	0.1144	-1.0395	ß	-2.2246	0.1452	1.1568	3.0896	1.6726	0.1769
	SE (±)	0.1925	0.3293	0.3293	0.2561	0.2722	0.4025	SE (±)	0.5000	1.1180	0.5839	0.5130	0.5501	0.7071
	Z	-1.637	2.656	-1.553	1.580	0.420	-2.582	Z	-4.449	0.130	1.981	6.023	3.040	0.250
	P	0.10159	0.00791	0.12033	0.11412	0.67421	0.00982	P	8.62e-06	0.89668	0.04757	1.71e-09	0.00236	0.80242
Zero Inflated														
	ß	0.1455	1.0725	-0.9721	0.4643	0.2578	-1.4396	ß	-2.2247	0.1453	1.3469	3.7100	2.4190	2.6907
	SE (±)	0.2810	0.4001	0.3880	0.3503	0.3796	1.0045	SE (±)	0.5000	1.1180	0.8484	0.5138	0.5984	0.8193
	Z	0.518	2.681	-2.505	1.325	0.679	-1.433	Z	-4.449	0.130	1.588	7.221	4.043	3.284
	P	0.60465	0.00734	0.01224	0.18509	0.49703	0.15181	P	8.63e-06	0.89658	0.11239	5.15e-13	5.29e-05	0.00102
Zero inflated w/ negative binomial dist														
	ß	-0.1028	1.2004	-0.7239	0.5127	0.2830	-1.2518	ß	-2.2247	0.1454	1.1569	3.5017	1.9572	2.2727
	SE (±)	0.3904	0.5761	0.4868	0.4466	0.4767	0.5394	SE (±)	0.5232	1.1762	0.6262	0.6047	0.7337	1.1292
	Z	-0.263	2.084	-1.487	1.148	0.594	-2.320	Z	-4.252	0.124	1.847	5.790	2.667	2.013
	P	0.7923	0.0372	0.1370	0.2509	0.5527	0.0203	P	2.12e-05	0.90160	0.06468	7.02e-09	0.00764	0.04414
Zero inflation														
	Observed zeros	104						Observed zeros	129					
	Predicted zeros	89						Predicted zeros	112					
	Ratio	0.86						Ratio	0.87					
	Prob 0 inflation	Yes						Prob 0 inflation	Yes					
Performance		Zero inflated	Poisson			Negative binomial distribution		Performance	Zero inflated	Poisson			Negative binomial distribution	
	AIC	412.554	427.983			410.662		AIC	337.838	391.428			320.634	
	Log scores	0.067	-1,202			0.067		Log scores	-0.907	-1.097			0.067	

Table S11 – Generalized Linear models used to assess the effect of bird species (HH – Black-winged stilt, RA – Pied avocet, CA – Kentish plover, SA – Little tern, IA – Audouin's gull, LM – Yellow-legged gull) on presence and number of plastics and their colours (Red, Brown) in faeces. Each model used is identified on the left. Associated ß±SE, Z and p values are presented. Highlighted in red are significant values (<0.05). At the bottom, zero inflation tests (ratio) and performance of the models performed on quantitative data are shown. Highlighted in red are the models selected in each case.

		Faeces - Colours												
		Red$Species							Brown$Species					
		CA	HH	IA	LM	RA	SA		CA	HH	IA	LM	RA	SA
Binomial - PRESENCE														
	ß	-2.1102	-15.4559	0.1643	-0.5978	0.3874	-0.5639	ß	-3.5835	1.6376	2.6452	1.3148	2.4441	0.9094
	SE (±)	0.5294	1398.7211	0.7523	0.9020	0.7183	0.9027	SE (±)	1.0138	1.4733	1.0874	1.1813	1.0921	1.2499
	Z	-3.986	-0.011	0.218	-0.663	0.539	-0.625	Z	-3.535	1.112	2.433	1.113	2.238	0.728
	P	6.73e-05	0.991	0.827	0.507	0.590	0.532	P	0.000408	0.266344	0.014986	0.265710	0.025228	0.466886
Poisson														
	ß	-1.8192	-15.4834	-0.2603	-0.2603	-0.2911	-1.6148	ß	-3.6109	1.5315	2.3424	1.5315	2.0603	0.8701
	SE (±)	0.4082	1226.0732	0.6455	0.6455	0.6455	1.0801	SE (±)	1.0000	1.4142	1.0541	1.1180	1.0690	1.2247
	Z	-4.456	-0.013	-0.403	-0.403	-0.451	-1.495	Z	-3.611	1.083	2.222	1.370	1.927	0.710
	P	8.35e-06	0.990	0.687	0.687	0.652	0.135	P	0.000305	0.278845	0.026269	0.170750	0.053948	0.477446
Zero Inflated														
	ß	-0.976944	-0.003545	-1.102485	1.442968	-1.133243	-2.456995	ß	-3.6088	1.5293	2.3402	3.1076	2.4588	0.8679
	SE (±)	0.971923	NaN	1.092994	1.168662	1.092993	1.394960	SE (±)	1.0753	1.4685	1.1258	1.4400	1.4522	1.2871
	Z	-1.005	NaN	-1.009	1.235	-1.037	-1.761	Z	-3.356	1.041	2.079	2.158	1.693	0.674
	P	0.3148	NaN	0.3131	0.2169	0.2998	0.0782	P	0.000791	0.297689	0.037642	0.030925	0.090429	0.500093
Zero inflated w/ negative binomial dist														
	ß	-0.977188	-0.007234	-1.102174	1.443146	-1.133055	-2.456746	ß	-3.6098	1.5303	2.3413	3.1087	2.4600	0.8689
	SE (±)	0.972048	NaN	1.093097	1.168779	1.093109	1.395883	SE (±)	1.0370	1.4407	1.0892	1.4116	1.4239	1.2552
	Z	-1.005	NaN	-1.008	1.235	-1.037	-1.760	Z	-3.481	1.062	2.149	2.202	1.728	0.692
	P	0.3148	NaN	0.3133	0.2169	0.2999	0.0784	P	0.000499	0.288140	0.031599	0.027652	0.084060	0.488752
Zero inflation														
	Observed zero	157						Observed zero	151					
	Predicted zero	155						Predicted zero	151					
	Ratio	0.99						Ratio	1.00					
	Prob 0 inflatio	No						Prob 0 inflatio	No					
Performance	Performance	Zero inflated	Poisson		Negative binomial distribution			Performance	Zero inflated	Poisson		Negative binomial distribution		
	AIC	136.446	133.010		138.446			AIC	156.317	146.294		158.317		
	Log scores	-0,325	0.073		-0,329			Log scores	-0,382	0.072		-0,386		

Table S12 – Generalized Linear models used to assess the effect of bird species (HH – Black-winged stilt, RA – Pied avocet, CA – Kentish plover, SA – Little tern, IA – Audouin's gull, LM – Yellow-legged gull) on presence and number of plastics and their shapes (fibre) and colours (blue) in regurgitates. Each model used is identified on the left. Associated ß±SE, Z and p values are presented. Highlighted in red are significant values (<0.05). At the bottom, zero inflation tests (ratio) and performance of the models performed on quantitative data are shown. Highlighted in red are the models selected in each

	Regurgitates										
	Total$Species				Fibre$Species				Blue$Species		
	IA	LM	SA		IA	LM	SA		IA	LM	SA
Binomial - Presence											
ß	-3.3499	1.8458	3.1021	ß	-4.0604	0.4361	3.6435	ß	-4.060	0.855	3.284
SE (±)	0.7194	0.7777	0.7571	SE (±)	1.0084	1.2370	1.0364	SE (±)	1.008	1.168	1.039
Z	-4.656	2.373	4.097	Z	-4.027	0.353	3.516	Z	-4.027	0.732	3.159
P	3.22e-06	0.0176	4.18e-05	P	5.66e-05	0.724429	0.000439	P	5.66e-05	0.46408	0.00158
Poisson											
ß	-2.6912	2.3728	4.0212	ß	-4.077	1.343	3.637	ß	-4.078	1.931	5.328
SE (±)	0.5000	0.5175	0.5036	SE (±)	1.000	1.095	1.011	SE (±)	1.000	1.054	1.002
Z	-5.382	4.585	7.985	Z	-4.078	1.226	3.599	Z	-4.078	1.832	5.318
P	7.35e-08	4.55e-06	1.41e-15	P	4.55e-05	0.220138	0.000319	P	4.55e-05	0.067	1.05e-07
Zero Inflated											
ß	0.4660	0.9003	1.6885	ß	-4.060	4.179	4.117	ß	-4.030	3.895	6.435
SE (±)	0.6490	0.6637	0.6517	SE (±)	1.728	1.850	1.742	SE (±)	2.475	2.534	2.475
Z	0.718	1.356	2.591	Z	-2.349	2.259	2.363	Z	-1.628	1.537	2.600
P	0.47270	0.17498	0.00958	P	0.0188	0.0239	0.0181	P	0.10343	0.12422	0.00933
Zero inflated w/ negative binomial dist											
ß	-1.1457	1.2544	2.4757	ß	-4.064	4.183	4.121	ß	-4.0773	1.9307	5.3281
SE (±)	1.3157	1.3879	1.3501	SE (±)	1.588	1.720	1.603	SE (±)	1.0845	1.1917	1.1490
Z	-0.871	0.904	1.834	Z	-2.559	2.432	2.570	Z	-3.759	1.620	4.637
P	0.3839	0.3661	0.0667	P	0.0105	0.0150	0.0102	P	0.00017	0.10520	3.53e-06
Zero inflation											
Observed zeros	161			Observed zeros	176			Observed zeros	179		
Predicted zeros	94			Predicted zeros	163			Predicted zeros	129		
Ratio	0.58			Ratio	0.96			Ratio	0.72		
Prob 0 inflation	Yes			Prob 0 inflation	No			Prob 0 inflation	Yes		
Performance	Zero inflated	Poisson	Negative binomial distribution		Zero inflated	Poisson	Negative binomial distribution		Zero inflated	Poisson	Negative binomial distribution
AIC	1346.631	1824.579	428.328	AIC	212.509	221.244	214.509	AIC	1105.039	1605.161	286.445
Log scores	-3.193	-4.351	0.055	Log scores	0.064	0.064	0.064	Log scores	-2.615	-3.826	0.057

Table S13 – Total amount of plastics from each category found in the bird species (HH – Black-winged stilt, RA – Pied avocet, CA – Kentish plover, SA – Little tern, IA – Audouin's gull, LM – Yellow-legged gull) and type of samples (faeces, regurgitates).

		Number of plastics found on each species								
		Faeces (n)						Regurgitates (n)		
Species		RA	HH	CA	SA	IA	LM	SA	LM	IA
Type										
	Fragment	7		5		5	20	229	26	
	Fibre	55	18	32	17	34	112	47	5	1
	Filament									
	Sfere									
	Film						0		22	3
	Ball			1			1		3	
Colour										
	Blue	27	14	27	8	14	35	255	3	1
	Green						2	1	17	
	Black	1	2		1	1	5		5	
	Purple	3					1	2		
	Transpare	19	1	4	4	11	76	4	14	2
	White				1		5	1	5	1
	Yellow									
	Red	5		6	1	4	4	3	8	
	Grey									
	Pink									
	Brown	7	1	1	2	9	4	10	4	
	Multicoulour						1			
Size										
	MesoPlast	1	1	2		1	9		44	4
	LargeMP	36	12	21	8	26	76	37	12	
	SmallMP	25	5	15	9	12	42	238		
	NanoPlastic						6			

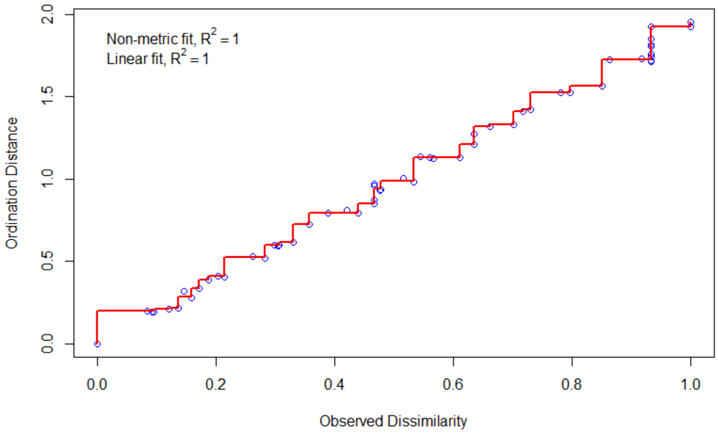


Figure S1– NMDS stressplot using “type” categories in faeces. Stressplot obtained from 50 iterations: k=2.

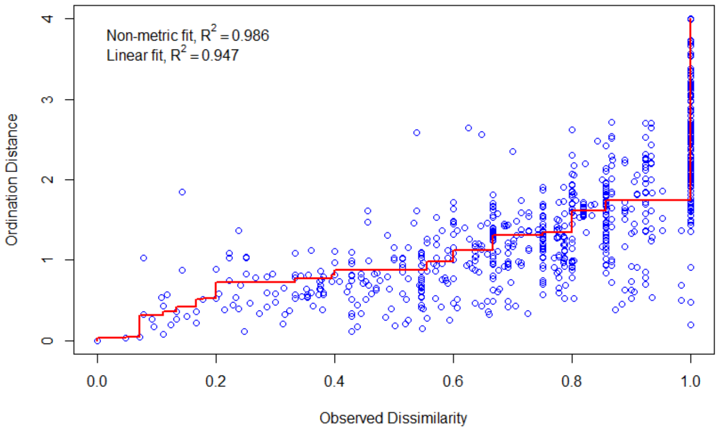


Figure S2 – NMDS stressplot using “colour” categories in faeces. Stressplot obtained from 50 iterations: k=2.

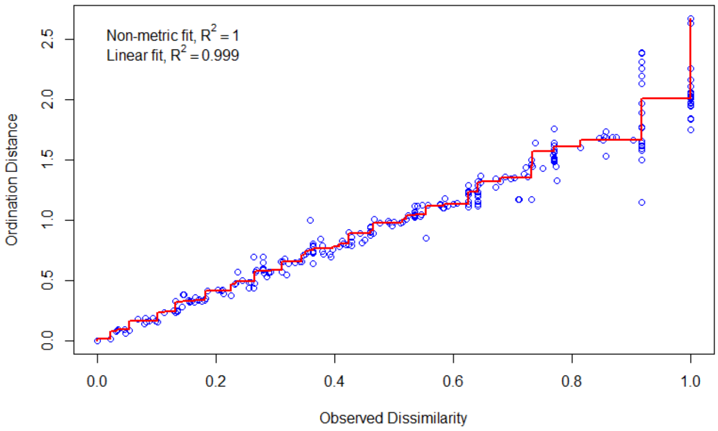


Figure S3 – NMDS stressplot using “size” categories in faeces. Stressplot obtained from 50 iterations: k=2.

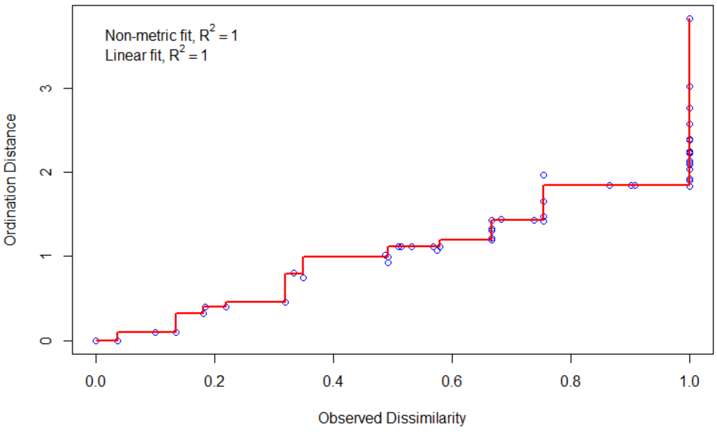


Figure S4 – NMDS stressplot using category type of the regurgitate. Stressplot obtained from 50 iterations: k=2.

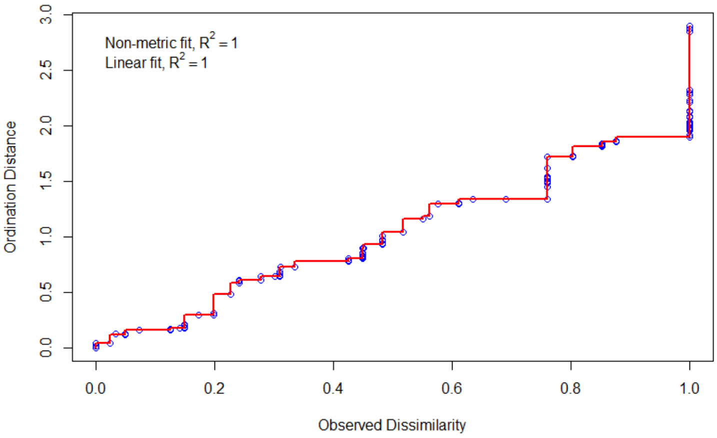


Figure S5 – NMDS stressplot using “colour” categories of the regurgitates. Stressplot obtained from 50 iterations: k=2.

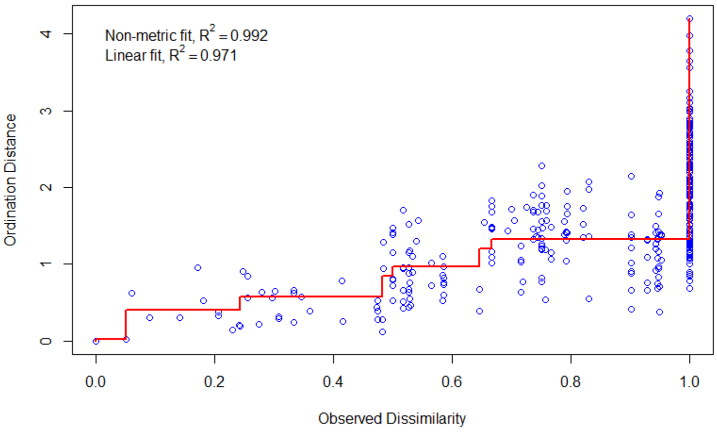
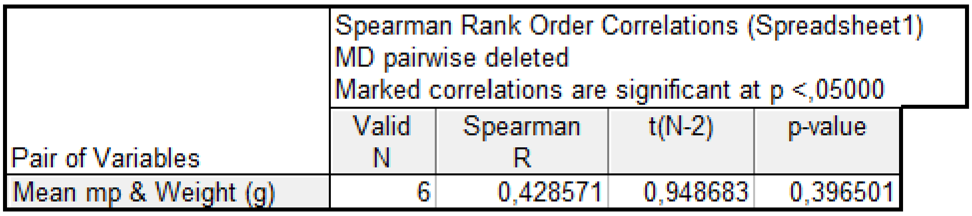


Figure S6 – NMDS Stressplot using “size class” categories of the regurgitate. Stressplot obtained from 50 iterations: k=2.

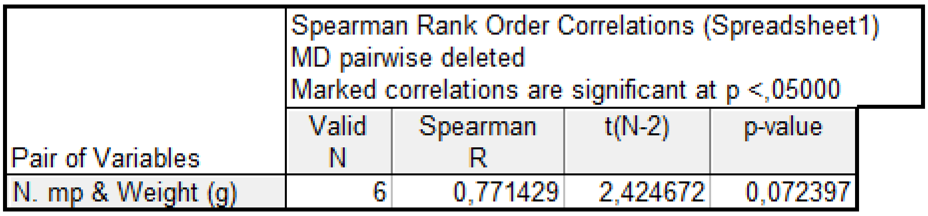
Table S13– Six-point Spearman correlation between the mean size of the microplastics found in faecal samples and the mean weight of the birds (extracted from literature). Spearman coefficient of 0.42 and p = 0.39 (>0.05).



Spearman Rank Order Correlations (Spreadsheet1)
MD pairwise deleted
Marked correlations are significant at p <,05000

Pair of Variables	Valid N	Spearman R	t(N-2)	p-value
Mean mp & Weight (g)	6	0,428571	0,948683	0,396501

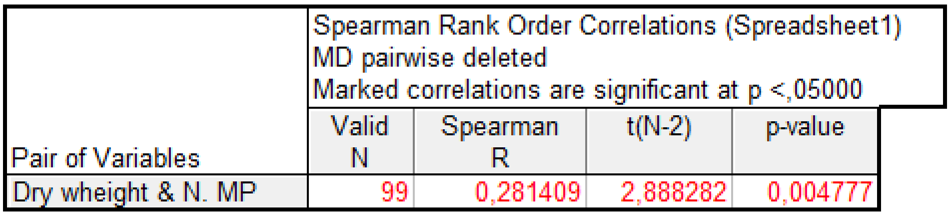
Table S14 – Six-point Spearman correlation between number of microplastics in faecal samples and the mean weight of the birds (extracted from literature). Spearman coefficient of 0.77 and p = 0.07 (>0.05).



Spearman Rank Order Correlations (Spreadsheet1)
MD pairwise deleted
Marked correlations are significant at p <,05000

Pair of Variables	Valid N	Spearman R	t(N-2)	p-value
N. mp & Weight (g)	6	0,771429	2,424672	0,072397

Table S15 – Spearman correlation between number of microplastics found in the faeces and associated faeces weight. Correlation done using only positive samples (samples with at least one microplastic) n=99 points, Spearman coefficient of 0.28 and p=0.004 (<0.05).



Spearman Rank Order Correlations (Spreadsheet1)
MD pairwise deleted
Marked correlations are significant at p <,05000

Pair of Variables	Valid N	Spearman R	t(N-2)	p-value
Dry wheight & N. MP	99	0,281409	2,888282	0,004777

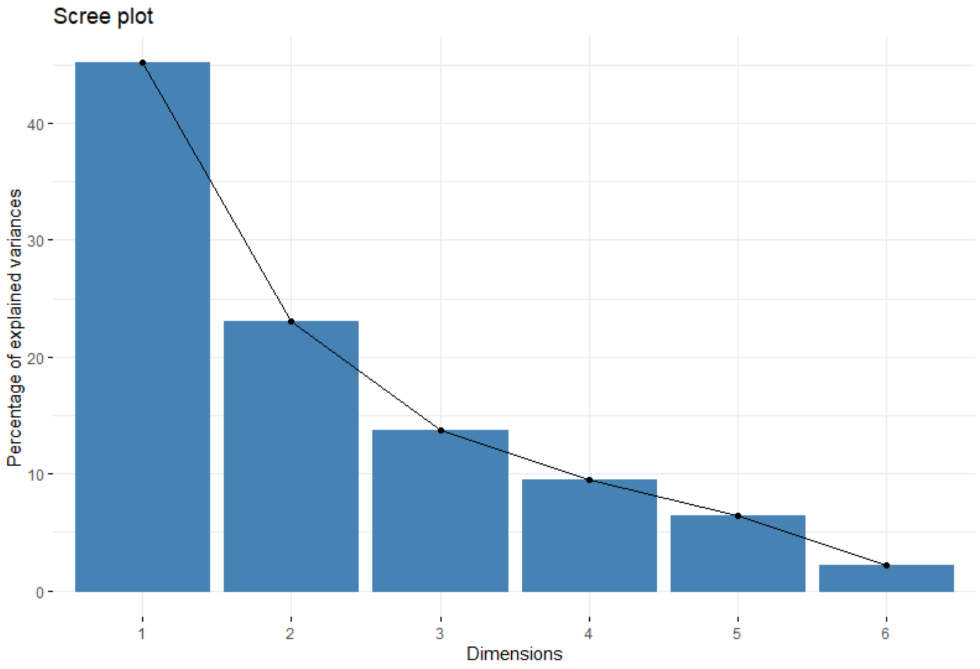
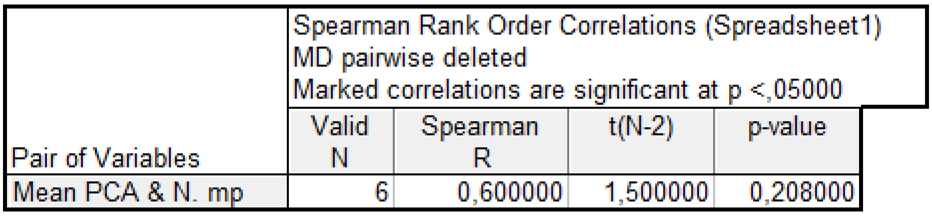


Figure S7 – Scree plot showing the amount of variation explained by each axis of the Principal component analysis. Axis 1 = 45.12; Axis 2 = 23.04; Axis 3= 13.69; Axis 4 = 9.43; Axis 5 = 6.46; Axis 6= 2.22.

Table S16 - Six-point Spearmann correlation between the number of microplastics in faecal samples and the mean score on Axis 1 of the PCA done using the concentrations of additives in eggs by bird species. Spearman coefficient of 0.60 and p = 0.20 (>0.05).



Spearman Rank Order Correlations (Spreadsheet1)
MD pairwise deleted
Marked correlations are significant at p <,05000

Pair of Variables	Valid N	Spearman R	t(N-2)	p-value
Mean PCA & N. mp	6	0,600000	1,500000	0,208000