



Research Paper

Fatty acids composition in yellow-legged (*Larus michahellis*) and lesser black-backed (*Larus fuscus*) gulls from natural and urban habitats in relation to the ingestion of anthropogenic materials



Catarina S. Lopes^{a,*}, Raquel C.C. Antunes^{b,a}, Vitor H. Paiva^a, Ana M.M. Gonçalves^{a,c}, Jorge J. Correia^b, Jaime A. Ramos^a

^a University of Coimbra, MARE – Marine and Environmental Sciences Centre, Department of Life Sciences, Calçada Martim de Freitas, 3004-517 Coimbra, Portugal

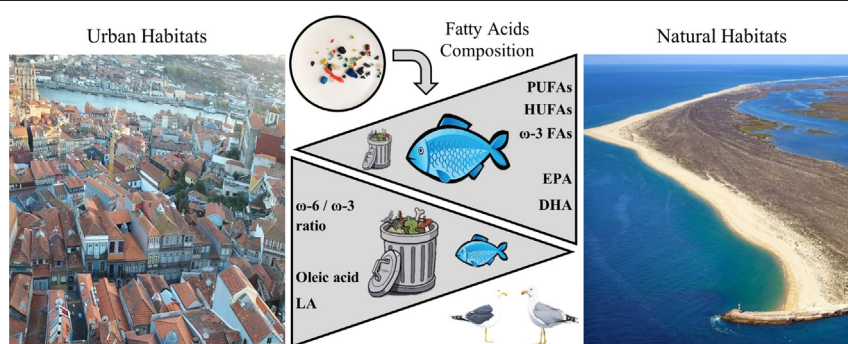
^b CIISA - Centre for Interdisciplinary Research in Animal Health, Faculty of Veterinary Medicine, University of Lisbon, Avenida da Universidade Técnica, 1300-477 Lisboa, Portugal

^c Department of Biology and CESAM, University of Aveiro, 3810-193 Aveiro, Portugal

HIGHLIGHTS

- Natural and urban gulls' fatty acids profiles were described to assess diet quality.
- Effects of ingesting debris on gulls' fatty acids composition were investigated.
- Physiologically important fatty acids were lower in the most urbanized location.
- Urban gulls rely on a diet with lower nutritional quality than natural ones.
- No effect of the ingestion of debris was found on gulls' fatty acids composition.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:

Received 30 June 2021

Received in revised form 12 October 2021

Accepted 16 October 2021

Available online 23 October 2021

Editor: Rafael Mateo Soria

Keywords:

Urbanization

Laridae

Diet analysis

Nutritional composition

Debris ingestion

Urban gulls

ABSTRACT

Urban habitats offer spatially and temporally predictable anthropogenic food sources for opportunistic species, such as several species of gulls that are known to exploit urban areas and take advantage of accessible and diverse food sources, reducing foraging time and energy expenditure. However, human-derived food may have a poorer nutritional quality than the typical natural food resources and foraging in urban habitats may increase birds' susceptibility of ingesting anthropogenic debris materials, with unknown physiological consequences for urban dwellers. Here we compare the fatty acids (FA) composition of two opportunistic gull species (the yellow-legged gull, *Larus michahellis*, and the lesser black-backed gull, *Larus fuscus*) from areas with different levels of urbanization, to assess differences in birds' diet quality among foraging habitats, and we investigate the effects of ingesting anthropogenic materials, a toxicological stressor, on gulls' FA composition. Using GC-MS, 23 FAs were identified in the adipose tissue of both gull species. Significant differences in gulls' FA composition were detected among the three urbanization levels, mainly due to physiologically important highly unsaturated FAs that had lower percentages in gulls from the most urbanized habitats, consistent with a diet based on anthropogenic food resources. The deficiency in omega (ω)-3 FAs and the higher ω -6: ω -3 FAs ratio in gulls from the most urbanized location may indicate a diet-induced susceptibility to inflammation. No significant differences in overall FA composition were detected between gull species. While we were unable to detect any effect of ingested anthropogenic materials on gulls' FA composition, these data constitute a valuable contribution to the limited FA literature in gulls. We encourage studies to explore the long-term physiological effects of the lower nutritional quality diet for urban dwellers, and to detect the sub-lethal impacts of the ingestion of anthropogenic materials.

© 2021 Elsevier B.V. All rights reserved.

* Corresponding author.

E-mail address: catarina.s.lopes90@gmail.com (C.S. Lopes).

1. Introduction

Over the years, population growth and consequent urbanization transformed natural coastal habitats into novel urban environments (Aronson et al., 2014; Marzluff, 2001) affecting ecosystems processes and dynamics (Oro et al., 2013), as well as animal physiology and behaviour (Rosenblatt and Schmitz, 2016). Features from the urban environment, such as air, light and noise pollution, put urban dwelling wildlife under stress that may result in several molecular and physiological changes including altered gene expression, endocrine modifications and increased oxidative stress (Partecke et al., 2006; Salmón et al., 2016; Watson et al., 2017). The existence of suitable breeding sites and the availability of food resources are crucial for animal populations to thrive and survive in urban conditions (Belant, 1997; Oro et al., 2013). Human-modified environments offer high, isolated and protected buildings that allow nesting in safer sites, without human disturbance, as well as abundant, predictable and readily available anthropogenic food, which attracts a multiple number of generalist and opportunistic animals, such as gulls, rats or foxes (Belant, 1997; Parra-Torres et al., 2020; Winton and River, 2017).

Urban-derived food may be easier to access when compared to natural sources (Bartumeus et al., 2010), allowing opportunistic species to reduce foraging time and energy expenditure (Furst et al., 2018; Zorroza et al., 2020). However, the increase in anthropogenic food subsidies may act as an ecological trap as human-derived food has typically a poorer nutritional quality than the natural food resources (Auman et al., 2008), which may lead to a reduced growth rate and body condition (Annett and Pierotti, 1999; Pierotti and Annett, 1991). Animals exploiting these locations to forage may be susceptible to a higher exposure to contaminants, poisoning and pathogen infections (Seif et al., 2018; Sorais et al., 2020; Yorio et al., 2020), as well as an increased probability of interacting with anthropogenic debris materials such as glass, fabric, metal, paper and especially plastics (Lopes et al., 2020, 2021). In fact, coastal and more generalist seabirds such as gulls are particularly exposed to such anthropogenic materials (Kühn and van Franeker, 2020; Lopes et al., 2021) and vulnerable to the direct deleterious impacts of their ingestion, which may include the obstruction to food passage, stomach ulcers and perforations of the gastrointestinal tract, disturbance in the assimilation of nutrients, damage to tissues, morbidity and starvation (Gregory, 2009; Henry et al., 2011; Kühn et al., 2015; Lavers et al., 2014; Ryan, 1987). In addition to these physical impacts, a range of less visible toxicological effects may be caused by the ingestion of anthropogenic materials, including a possible exposure to hazardous chemicals, especially from plastics containing known or suspected endocrine disrupting chemicals as additives (Gallo et al., 2018) which might contribute to neurological, endocrine and reproductive complications, and ultimately to death (Bouland et al., 2012; Rochman et al., 2016). In fact, examining toxicological effects of the ingestion of anthropogenic materials is difficult and evidence about the transfer of chemicals between plastics and animals is ambiguous. Most studies report that plastic ingestion may contribute to a higher exposure of 'plastic-adhered pollutants' (Lavers and Bond, 2016; Tanaka et al., 2013; Yamashita et al., 2011; also see Herzke et al., 2016; Provencher et al., 2018; Roman et al., 2019). Yet, this transfer of chemicals may be bidirectional and also occur from the animal to the plastic particles, with such particles acting as "cleaning" factors and reducing the chemicals that are already present in the animal (Thaysen et al., 2020). The toxicological effects of ingested anthropogenic materials and whether they are a source or sink of chemicals to bird species are complex and dependent on the species' ecological context (e.g. exposure level and feeding ecology, Thaysen et al., 2020).

Large gulls *Laridae*, among them the yellow-legged gull (YLG; *Larus michahellis*) and the lesser black-backed gull (LBBG; *Larus fuscus*) have become more common in urban areas, with established breeding populations around the world, benefiting from a more temperate and stable microclimate and fewer natural predators than in natural habitats

(Auman et al., 2008; Huig et al., 2016; Méndez et al., 2020; Pais de Faria et al., 2021a; Spelt et al., 2019). As opportunistic foragers, gulls use a wide variety of foraging habitats and strategies, being capable of exploiting different food types, especially anthropogenic food remains collected in landfills and within urban habitats (Gyimesi et al., 2016; Matos et al., 2018; Pais de Faria et al., 2021b; Parra-Torres et al., 2020; Ramos et al., 2009; Spelt et al., 2019). This resulted in an increase of their urban population numbers over the last few years (Duhem et al., 2008; Nager and O'Hanlon, 2016; Vidal et al., 1998). Foraging gulls are known to ingest anthropogenic materials when foraging at their natural habitats (review by Battisti et al., 2019) and at urban areas and landfills (Lopes et al., 2021). Yet, possible invisible physiological effects that may arise from ingesting those materials are poorly known as it may not result in birds' death but in a poorer health condition, possibly only detectable at molecular and cellular organization levels (Lavers et al., 2019; Roman et al., 2019). Many impacts from the exposure to plastics and other anthropogenic materials are perceived, but regarding subtle effects not all perceived impacts are truly demonstrated, measured and supported by evidence, and even fewer are empirically verified in realistic exposure scenarios (Koelmans et al., 2017; Rochman et al., 2016). Therefore, sub-lethal impacts of the ingestion of anthropogenic materials may be difficult to detect and may suffer from confounding bias (Roman et al., 2021), as factors other than debris ingestion might influence the observed effects at the individual level (Rochman et al., 2016). Generally, birds capable to survive and even thrive in urbanized areas are known to experience behavioral and physiological adaptations (Isaksson et al., 2015; Partecke et al., 2006; Shochat et al., 2010). Despite the known capability of gulls to exploit urban habitats and human-derived food resources, little is known about the associated physiological consequences of doing so, and if there are any consequences to their physiology from the ingestion of anthropogenic materials.

Fatty acids (hereafter FA) are the largest constituent of lipids (e.g. triglycerides, phospholipids and wax esters) which have different metabolic functions within an animal's body from storage of energy to structural components of cell membranes (Williams and Buck, 2010). FAs are obtained *via* dietary sources or by *de novo* biosynthesis, however, as birds are only capable of synthesising certain FAs, the majority of birds' FAs are acquired through their diet and, therefore, FA signatures of storage tissues largely reflect diet (Williams and Buck, 2010). FA analysis has been used to assess birds' diet quality and to examine differences or changes in foraging patterns and/or diets both within and between populations of predator species (Iverson et al., 2007; Karnovsky et al., 2012; Wang et al., 2009). Recently, the potential of using FA composition as a response to toxicological factors has been explored to assess the sub-lethal impacts of plastic ingestion in seabirds (in Procellariiforms, Puskic et al., 2019), after some reports of a negative correlation between ingested plastic and fat deposition in seabirds (Auman et al., 1997; Connors and Smith, 1982).

FA signatures of fledgling gulls are known to differ between urban and natural habitats (Pais de Faria et al., 2021b), however, variation in FA composition has rarely been investigated in the context of urbanization, with the exception of passerines (e.g. Andersson et al., 2015; Isaksson et al., 2017). Polyunsaturated (PUFAs) and highly unsaturated fatty acids (HUFAs) are especially relevant to characterize as they are involved in regulating birds' physiological processes (Watson et al., 2017). These FAs are strictly dietary (i.e. essential fatty acids, EFAs) for all birds, mainly obtained by feeding on aquatic prey (e.g. fish; Gladyshev et al., 2009), and can affect some aspects of birds' performance (Twining et al., 2018). The ratio omega (ω)-6: ω -3 FAs is also interesting to assess in urbanization studies as it is related with inflammatory responses and oxidative stress (Isaksson, 2015; Romieu et al., 2008). A high total of this ratio is associated with increased sensitivity to antigens by promoting inflammatory reactions and oxidative stress (Romieu et al., 2008). Overall, the FA composition of blood and tissues can play an important role on birds' health in urban habitats.

In this study we aim to 1) compare the FA composition of two gull species (YLG and LBBG) from three wildlife rescue centres that receive gulls from areas with different levels of urbanization, and to 2) investigate if there is any effect of ingesting anthropogenic materials on FA composition. We predict that FA composition will differ between urban and natural dwellers (i.e. between individuals from different wildlife rescue centres) mainly due to differences in diet between urban and natural habitats. As urban dwellers forage more on anthropogenic food resources than on marine prey, when compared to natural dwellers, we expect individuals from the most urbanized area to exhibit lower percentages of ω -3 PUFAs and HUFAs and a higher ω -6: ω -3 FAs ratio. As both species are known to be generalist and to forage on similar anthropogenic food subsidies, we do not anticipate major differences in overall FA composition between both gull species. We suggest that differences in diet among habitats should be the main driver for the possible differences in FA composition, however, differences in FA profiles may be also a response to toxicological stressors such as the ingestion of anthropogenic materials that may disrupt nutritional pathways. Despite the difficulty in detecting sub-lethal impacts from the exposure to anthropogenic materials, we predict that their ingestion, if it occurs at high and toxic levels, could have physiological consequences for gulls and thus their FA composition should present differences as a response to this toxicological stress.

2. Material and methods

2.1. Study sites and sampling processing

This study analysed 47 individuals from both yellow-legged (YLG, *Larus michahellis*, $n = 23$) and lesser black-backed gulls (LBBG, *Larus fuscus*, $n = 24$). All individuals used in this study were found stranded as a result of injury, illness or exhaustion, and brought by national authorities (Institute of Nature Conservation and Forests, ICNF) or by locals to one of the three wildlife rescue centres considered for this study, located across Portugal: Centro de Recuperação do Parque Biológico de Gaia (PBGaia, $41^{\circ} 05' 52''$ N, $8^{\circ} 33' 23''$ W, $n = 12$), Centro de Recuperação de Animais Silvestres de Lisboa (LxCRAS, $38^{\circ} 44' 24''$ N, $9^{\circ} 11' 11''$ W, $n = 15$) and Centro de Recuperação e Investigação de Animais Selvagens (RIAS, $37^{\circ} 02' 03''$ N, $7^{\circ} 48' 47''$ W, $n = 20$, Fig. 1). These three rescue centres serve distinct areas of the country, with different characteristics, and animals entering these wildlife centres should be experiencing different habitats and distinct levels of urbanization prior to their admission. In addition to other areas, PBGaia mostly serves the Metropolitan Area of Porto, where Porto is the second largest city of Portugal (PORDATA, 2011) that lies on the right side of the mouth of the Douro River, close to sea. A known population of urban gulls dwell in the city of Porto throughout the year, using certain areas of the city to rest (Pais de Faria et al., 2021a), and public and private buildings to construct their rooftop nests (Lopes et al., 2020). On the contrary, RIAS serves mostly the Ria Formosa Natural Park which has five barrier sandy islands and two peninsulas that form a narrow strip of dunes that separate the lagoon from the Atlantic Ocean (Ceia et al., 2010), and is located relatively far from metropolitan and populated urban centres. For this study, all studied gulls from PBGaia were collected in the urban metropolitan area of Porto, and all studied gulls from RIAS were collected in natural areas of the Ria Formosa Natural Park. LxCRAS, in turn, serves not only the metropolitan area of Lisbon but also the natural breeding and resting areas around the city. Thus, gulls entering this recovery centre should either come from the breeding population of the metropolitan area of Lisbon or from natural colonies such as Berlenga Island ($39^{\circ} 24' 49''$ N, $9^{\circ} 30' 29''$ W), from which individuals are known to forage over fisheries leftovers at the seashore south of Lisbon (Ceia et al., 2014). Necropsies were performed, preferably, on recently dead animals, which either died right before or after admission (~60% of the total necropsied gulls), followed by individuals with the shortest hospitalization time possible, never longer than 2 weeks. Such selection of

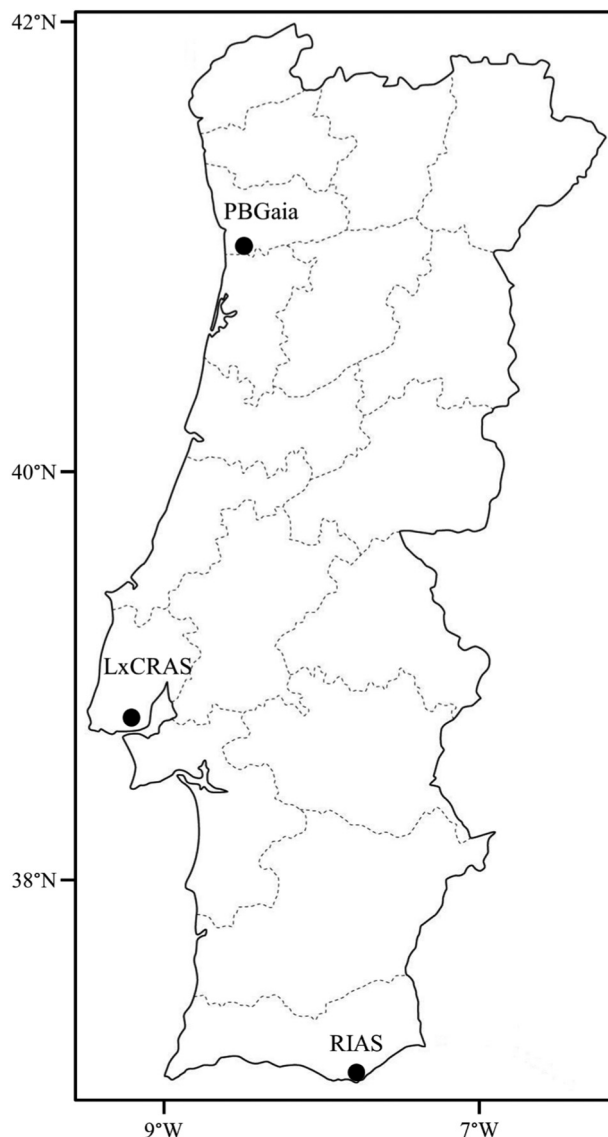


Fig. 1. Location of the Portuguese wildlife rescue centres from where gulls were sampled: Centro de Recuperação do Parque Biológico de Gaia (PBGaia), Centro de Recuperação de Animais Silvestres de Lisboa (LxCRAS) and Centro de Recuperação e Investigação de Animais Selvagens (RIAS).

individuals intends to reflect the conditions of the environment in which gulls dwelled (i.e. urban vs. natural locations) as much as possible, rather than conditions at each rescue centre. All individuals were collected between September 2019 and January 2020, each bird was labelled and kept frozen at -20°C until dissection, and necropsies were performed in November 2019 at RIAS, January 2020 at PBGaia and March and May 2020 at Anatomical Pathology Laboratory, Faculty of Veterinary Medicine, University of Lisbon (FMV-UL, individuals from LxCRAS).

Necropsies were performed following the dissection techniques of Peleteiro (2016) and van Franeker (2004). Whenever possible, data on body condition, probable cause of death (clinical history), body weight, age and sex were recorded for each individual. Body condition score (BCS) was recorded based on the pectoral muscle condition, assessed by its palpation using a scale of 1 (cachexic/lean) to 5 (obese). Probable cause of death was determined considering clinical history, clinical signs and/or necropsy findings for each individual, and gulls were diagnosed with gulls' paretic syndrome, trauma or unknown causes of death. Paretic syndrome affecting gulls in coastal Portugal has undetermined

causes, outbreaks occur mainly in September and October each year, and results in gulls' inability to fly, diarrhoea, paresis, dyspnoea, stiffness of neck and dehydration (Costa et al., 2021). Individuals chosen for this study had identical degrees of the disease, with similar symptoms. Trauma category included gulls that presented fractured bones (mainly wing bone fractures), articular dislocations and open wounds most likely linked with human-related collisions (e.g. cars, boats) during their foraging activities in fishing harbours and urbanized areas. Sex was determined through direct observation of the reproductive tract at the celomic cavity and age was recorded as adult (more than 3 years old) or immature (1–3 years) gulls, based on their plumage evaluation. All individuals were weighted on an electronic balance to the nearest 1 g.

Birds' entire digestive system (mouth, proventriculus, gizzard, intestines and cloaca) was carefully examined for the presence of plastics and other anthropogenic materials (glass, wood, rubber, fabric, etc.). Visible anthropogenic items (>1 mm) were collected and washed in a glass petri dish with saline solution. These materials were stored in tubes with saline solution and properly labelled per bird and the respective location on the digestive system, until further analysis.

In the laboratory, anthropogenic items were left at room temperature until they were completely dry. Items were sorted, counted and categorized into several categories of materials: plastic, glass, wood, metal, fabric, rubber and paper (adapted from Provencher et al., 2017). As the last four categories were found in a small number of samples, to simplify they were grouped in a "other" category. Plastics were also sub-divided in four different types: sheetlike (e.g. plastic bags and cling film), threadlike (e.g. fishing lines, plastic strings, and ribbons), fragments (unidentifiable fragments from the break-up of larger plastic items as well as intact items), and foamed plastics (e.g. styrofoam). Items' colours were also noted following Provencher et al. (2017) and included the categories: white/clear, yellow, green, blue/purple, red/pink, brown/orange, grey/silver, black and more than one colour. The biggest axis of each item was measured using graph paper, with an accuracy of 0.5 mm. Debris items were weighted per individual and per category to the nearest 0.0001 g using a precision balance.

2.2. FA quantification

From each necropsied bird, a sample of subcutaneous adipose tissue from the breast, specifically from the interior side of the pelvic limb, was collected. Fat tissues were stored in microtubes with alcohol 70% covering the sample, individually labelled and stored at -4°C . Fat tissues were then dried and weighted (0.03–0.6 g) and submitted to the FA extraction protocol. The extraction of total lipids and methylation to fatty acid methyl esters (FAMES) was performed following the methodology described by Gonçalves et al. (2012). Samples were incubated with methanol for the extraction of lipids. The nonadecanoic acid (C19:0, Fluka 74208) was added as an internal standard for further quantification. Samples were centrifuged and vacuum dried. FAMES identification was carried out through Gas Chromatography-Mass Spectrometry (GC-MS), using a Thermo Scientific Trace 1310 Network (Waltham, MA, USA) equipment, equipped with TR-FFAP (Ton Refrigeration Free Fatty Acid Phase) column of 0.32 mm internal diameter (i.d.), 0.25 μm film thickness, and 30 m long. The sample was injected at an injector temperature of 250°C , lined with a split glass liner of 4.0 mm i.d. The initial oven temperature was 80°C , followed by three ramps of linear temperature increase: $25^{\circ}\text{C min}^{-1}$ until 160°C ; $2^{\circ}\text{C min}^{-1}$ until 210°C and finally an increase of $40^{\circ}\text{C min}^{-1}$ until a final temperature of 230°C was reached and maintained for 10 min. Helium was used as carrier gas at a flow rate of 1.4 mL min^{-1} . A Thermo Scientific ISQ 7000 Network Mass Selective Detector at scanning m/z ranges specific for fatty acids in Selected Ion Monitoring (SIM) mode acquisition was used. The detector starts operating 3.5 min after injection, corresponding to solvent delay. The injector ion source and transfer line were maintained at 240°C and 230°C , respectively. Integration of FAME peaks were carried

out using the equipment's software. Identification of each peak was performed by retention time and mass spectrum of each FAME, comparing to the Supelco®37 component FAME mix (Sigma-Aldrich, Steinheim, Germany). Finally, each peak area was extracted and then quantified as $\mu\text{g/g}$.

2.3. Statistical analysis

Each FA of the gulls' adipose tissue, initially in abundances ($\mu\text{g/g}$), was converted to a percentage of the total FAs, per individual.

Firstly, general linear models (GLMs) with Gaussian family and identity link were performed to evaluate the effect of the wildlife rescue centre (PBGaia, LxCRAS and RIAS) and species (YLG and LBBG) on the percentages of FA groups (SFAs, MUFAs, PUFAs, HUFAs, ω -3 and ω -6) and on the total ω -6/ ω -3 ratio. When the main effect of rescue centre or species was significant in the model, we proceeded by traditional post-hoc Tukey tests.

To normalize FAs percentages data, we used the arcsine transformation. To analyse the effect of gulls' characteristics (wildlife rescue centre, species, age, sex, body condition score and clinical history) on their FA composition, we used partial least squares discriminant analysis (PLS-DA), a supervised multi-dimensional statistical model analysis that focuses on covariance while reducing dimensionality and takes into consideration both dependent and independent variables (Hadi and Ling, 1998). PLS-DA were performed using all transformed FA percentages independently of their origin (dietary or non-dietary) and the number of double-bonds (saturated or unsaturated FA).

To understand how the number of anthropogenic materials per individual (number of items) differed between wildlife rescue centres and species, we performed zero inflated models, with negative binomial distributions to account for overdispersion. Models were performed considering the total number of items per individual (all debris) and the total number of plastic items per individual (all plastic). Zero inflated models use a reference category against which the remaining data is compared, thus, PBGaia was assigned as the reference rescue centre and LBBG was assigned as the reference species.

Mass of ingested anthropogenic materials was \log_{10} transformed to attain normality. General linear models (GLMs) with Gaussian family and identity link function were performed to evaluate the effect of wildlife rescue centre and species in the mass of ingested anthropogenic materials.

The relationship between ingested anthropogenic materials' mass and number of items on YLG and LBBG body mass was investigated using linear regression. A Cook's distance of >3 identified one statistical outlier that was excluded from this analysis (Rousseeuw and Leroy, 2005). To analyse possible patterns of ingested anthropogenic materials' mass effects on FA composition, we performed partial least squares regression (PLSR), also with all transformed FA percentages independently of their origin (dietary or non-dietary) and the number of double-bonds (saturated or unsaturated FA).

The R statistical program (R Core Team, 2019) was used in all analyses, with a significance level of $p < 0.05$. GLM models were performed using MASS R package (Venables and Ripley, 2002) and post hoc tests were performed using lsmeans R package (Lenth, 2016). Zero-inflated models were performed using pscl R package (Zeileis et al., 2008; Jackman, 2017). PLS-DA and PLSR were performed using mixOmics R package (Rohart et al., 2017).

3. Results

3.1. FA composition among wildlife rescue centres and species

A total of 23 FAs were found and quantified in the adipose tissue of YLG and LBBG from the three wildlife rescue centres (Table 1). Monounsaturated FAs (MUFAs) were the predominant FA group accounting for, on average, 48.1% of all FAs, ranging from 44.2% (for LBBG in RIAS) to

Table 1

Relative abundance of adipose tissue fatty acids (% of the total fatty acid content) in two gull species (yellow-legged gull, YLG, *Larus michahellis* and lesser black-backed gull, LBBG, *Larus fuscus*) from three wildlife rescue centres (Centro de Recuperação do Parque Biológico de Gaia, PBGaia; Centro de Recuperação de Animais Silvestres de Lisboa, LxCRAS and Centro de Recuperação e Investigação de Animais Selvagens, RIAS). Individuals from PBGaia represent the most urbanized gull population, gulls from RIAS represent the most natural individuals and LxCRAS individuals are both natural and urban dwellers. Data is presented as means \pm standard error of the means (SEM). C:D = number of carbon atoms:double bonds; N = number of individuals with that fatty acid detected in their adipose tissue (for Total SFA, Total MUFA, Total PUFA and Total HUFA: N = diversity of FAs per wildlife rescue centre and species, in italics); LA = linoleic acid; α LNA = α -linolenic acid; DGLA = dihomo- γ -linolenic acid; ARA = arachidonic acid; EPA = eicosapentaenoic acid; DHA = docosahexaenoic acid; SFA = saturated fatty acids; MUFA = Monounsaturated fatty acids; PUFA = Polyunsaturated fatty acids; HUFA = Highly unsaturated fatty acids.

FA	C:D	PBGaia (n = 12)				LxCRAS (n = 15)				RIAS (n = 20)			
		YLG (n = 10)		LBBG (n = 2)		YLG (n = 4)		LBBG (n = 11)		YLG (n = 9)		LBBG (n = 11)	
		N	Mean \pm SEM	N	Mean \pm SEM	N	Mean \pm SEM	N	Mean \pm SEM	N	Mean \pm SEM	N	Mean \pm SEM
Lauric acid	C12:0	9	0.82 \pm 0.15	1	0.28 \pm 0.28	2	0.43 \pm 0.27	9	0.36 \pm 0.09	4	0.37 \pm 0.19	4	0.27 \pm 0.16
Tridecyl acid	C13:0	0		0		0		2	0.02 \pm 0.02	1	0.01 \pm 0.01	1	0.01 \pm 0.01
Myristic acid	C14:0	10	2.59 \pm 0.31	2	1.37 \pm 0.29	4	4.27 \pm 1.38	11	4.51 \pm 0.95	9	5.49 \pm 0.72	11	4.67 \pm 0.61
Pentadecyl acid	C15:0	10	1.13 \pm 0.18	2	0.95 \pm 0.26	4	0.64 \pm 0.14	11	0.94 \pm 0.14	9	1.01 \pm 0.14	11	0.77 \pm 0.09
Palmitic acid	C16:0	10	20.27 \pm 2.32	2	22.15 \pm 4.88	4	22.84 \pm 0.74	11	22.67 \pm 1.13	9	22.76 \pm 1.94	11	21.75 \pm 0.84
Margaric acid	C17:0	10	0.4 \pm 0.03	2	0.24 \pm 0.02	4	0.57 \pm 0.11	11	0.61 \pm 0.07	9	0.76 \pm 0.09	11	0.65 \pm 0.06
Stearic acid	C18:0	10	9.53 \pm 0.72	2	10.79 \pm 0.34	4	8.46 \pm 1.2	11	8.13 \pm 0.63	9	7.86 \pm 0.32	11	8.46 \pm 0.79
Arachidic acid	C20:0	10	0.37 \pm 0.05	2	0.49 \pm 0.2	4	0.37 \pm 0.11	11	0.38 \pm 0.06	9	0.6 \pm 0.12	11	0.4 \pm 0.07
Behenic acid	C22:0	1	0.03 \pm 0.03	0		0		0		0		1	0.01 \pm 0.01
Tricosyl acid	C23:0	1	0.09 \pm 0.09	0		0		1	0.07 \pm 0.07	0		1	0.24 \pm 0.24
Total SFA		9	35.24 \pm 2	7	36.27 \pm 5.31	7	37.59 \pm 1.14	9	37.7 \pm 1.49	8	38.86 \pm 2.78	10	37.24 \pm 1.42
Palmitoleic acid	C16:1n-7	10	6.01 \pm 1.61	2	2.3 \pm 0.5	4	7.04 \pm 2.44	11	6.69 \pm 1.13	9	8.66 \pm 1.16	11	7.8 \pm 0.75
Heptadecenoic acid	C17:1n-10	8	0.33 \pm 0.07	1	0.08 \pm 0.08	3	0.51 \pm 0.24	10	0.47 \pm 0.07	8	0.7 \pm 0.11	11	0.65 \pm 0.08
Oleic acid	C18:1n-9	10	43.34 \pm 3.02	2	50.17 \pm 0.27	4	31.04 \pm 4	11	34.62 \pm 3.73	9	26.1 \pm 2.58	11	30.04 \pm 2.55
Eicosenoic acid	C20:1n-9	10	1.57 \pm 0.28	2	1.7 \pm 0.14	4	3.36 \pm 0.8	11	3.85 \pm 0.99	9	5.14 \pm 0.96	11	3.28 \pm 0.54
Cetoleic acid	C22:1n-11	5	0.75 \pm 0.33	0		4	2.45 \pm 0.72	9	3.33 \pm 1.25	9	7.26 \pm 3.35	9	2.34 \pm 0.54
Nervonic acid	C24:1n-9	0		0		1	0.23 \pm 0.23	0		0		2	0.11 \pm 0.08
Total MUFA		5	51.99 \pm 2.13	4	54.25 \pm 0.71	6	44.63 \pm 1.13	5	48.97 \pm 3.06	5	47.85 \pm 2.39	6	44.22 \pm 2.24
LA (ω -6)	C18:2n-6	10	10.5 \pm 1.86	2	9.1 \pm 5.64	4	7.67 \pm 3.38	11	5.96 \pm 0.85	9	4.68 \pm 0.95	11	6.41 \pm 1.21
α -LNA (ω -3)	C18:3n-3	4	0.13 \pm 0.06	0		3	0.6 \pm 0.21	6	0.42 \pm 0.18	6	0.54 \pm 0.16	10	0.64 \pm 0.13
Eicosadienoic acid (ω -6)	C20:2n-6	2	0.09 \pm 0.07	0		3	0.25 \pm 0.09	6	0.28 \pm 0.12	5	0.15 \pm 0.05	8	0.26 \pm 0.05
DGLA (ω -6)	C20:3n-6	0		0		0		0		0		1	0.04 \pm 0.04
Total PUFA		3	10.72 \pm 1.94	1	9.1 \pm 5.64	3	8.52 \pm 3.46	3	6.66 \pm 0.81	3	5.37 \pm 0.94	4	7.34 \pm 1.15
ARA (ω -6)	C20:4n-6	3	1.83 \pm 1.76	1	0.38 \pm 0.38	4	1.92 \pm 1.02	7	1.13 \pm 0.62	7	0.99 \pm 0.5	9	1.9 \pm 1.3
EPA (ω -3)	C20:5n-3	1	0.04 \pm 0.04	0		3	2.14 \pm 1.02	5	1.34 \pm 0.76	7	1.57 \pm 0.57	10	2.25 \pm 0.76
DHA (ω -3)	C22:6n-3	1	0.18 \pm 0.18	0		3	5.2 \pm 2.42	6	4.2 \pm 1.96	8	5.36 \pm 1.37	10	7.04 \pm 2.06
Total HUFA		3	2.05 \pm 1.98	1	0.38 \pm 0.38	3	9.26 \pm 3.7	3	6.67 \pm 2.89	3	7.92 \pm 2.1	3	11.2 \pm 3.04
Mean number of FAs/individual			13.5 \pm 0.54		11.5 \pm 0.5		16.5 \pm 0.96		15.55 \pm 0.85		16.11 \pm 0.68		17 \pm 0.47

54.3% (for LBBG in PBGaia, Fig. 2A). This was particularly due to the high percentages of the oleic acid (C18:1n-9) in all individuals (Table 1). The second most abundant FA group was the saturated FAs (SFAs) that accounted for, on average, 37.2% of all FAs, ranging from 35.2% (for YLG in PBGaia) to 38.9% (for YLG in RIAS, Fig. 2A). PUFAs (polyunsaturated FAs) presented higher percentages than HUFAs (highly unsaturated FAs) in gulls from PBGaia (9.1–10.7% vs. 0.38–2.1%, respectively), but this did not occur in individuals from LxCRAS (6.7–8.5% PUFAs vs. 6.7–9.3% HUFAs) nor from RIAS (5.4–7.3% PUFAs vs. 7.9–11.2% HUFAs, Table 1, Fig. 2A). Individuals from PBGaia presented the lowest percentage of ω -3 FAs (0% for LBBG and 0.4% for YLG vs. a range of ~6% in LxCRAS to ~10% in RIAS, both for LBBG, Fig. 2B). On the contrary, ω -6 FAs presented the highest percentage for YLGs from PBGaia (12.4%, Fig. 2B), but the range of detected ω -6 FAs percentages was not so wide as that of ω -3 FAs (range of 5.8% in RIAS to 9.8% in LxCRAS, both for YLG, Fig. 2B). The total ω -6/ ω -3 ratio was the highest for PBGaia YLGs (38.8), and the lowest for RIAS individuals (1.8 for YLG and 4.7 for LBBG, Fig. 2B).

GLM results testing the effect of wildlife rescue centre and species on the percentages of FAs groups (SFAs, MUFAs, PUFAs, HUFAs, ω -3 and ω -6) and the total ω -6/ ω -3 ratio showed that the percentage of all FA groups as well as the total ω -6/ ω -3 ratio did not vary significantly among species ($F < 1.58$; $p > 0.22$, Table 2A). SFAs, MUFAs and ω -6 also did not vary among rescue centres ($F < 2.77$; $p > 0.07$), but PUFAs, HUFAs and ω -3 were significantly different among rescue centres ($F > 3.23$; $p < 0.05$, Table 2A), more specifically between PBGaia and RIAS (all Tukey $p < 0.045$, Table 2B). The total ω -6/ ω -3 ratio was also different among rescue centres ($F_{2, 33} = 17.7$; $p < 0.001$, Table 2A), in particular between PBGaia

and RIAS and between PBGaia and LxCRAS (all Tukey $p < 0.001$, Table 2B). RIAS and LxCRAS did not present significant differences for the ratio ω -6/ ω -3 (Table 2B).

Gulls from PBGaia had a lower diversity of FAs, i.e. a lower number of FAs per individual (Table 1), than gulls from RIAS and LxCRAS. We identified 5 FAs that individually accounted for >7% of the total FAs composition: C18:1n-9 (ranging from 26.1% for YLG in RIAS to 50.2% in PBGaia for LBBG), C16:0 (ranging from 20.3% in PBGaia to 22.8% in LxCRAS, both for YLG), C18:0 (ranging from 7.9% in RIAS for YLG to 10.8% in PBGaia for LBBG), C16:1n-7 (ranging from 2.3% in PBGaia to 7.8% in RIAS, both for LBBG) and C18:2n6 (ranging from 4.7% in RIAS to 10.5% in PBGaia, both for YLG, Table 1).

3.2. Influence of gulls' characteristics on FA composition

From the 47 necropsied gulls, there were more immature individuals than adults (34 immature vs. 13 adults) but sex was evenly distributed (23 female vs. 20 male gulls; the sex of 4 gulls was impossible to determine). Considering the probable cause of death, 26 gulls died from paretic syndrome complications and 18 from trauma lesions (3 gulls had unknown causes of death). Overall, 2 was the most common BCS recorded (22 gulls), followed by BCS = 3 (12 gulls) and BCS = 1 (7 gulls, Table S1).

A PLS-DA was run on all the percentages (arcsine transformed) of the FAs detected in the adipose tissue samples (Fig. 3) with wildlife rescue centre as response variable. Components (comp) 1, 2 and 3 accounted for 30%, 13% and 13% of the variation in the data, respectively (Table S2). The three wildlife rescue centres grouped distinctly; in particular PBGaia (orange ellipse in Fig. 3) was clearly separated from the

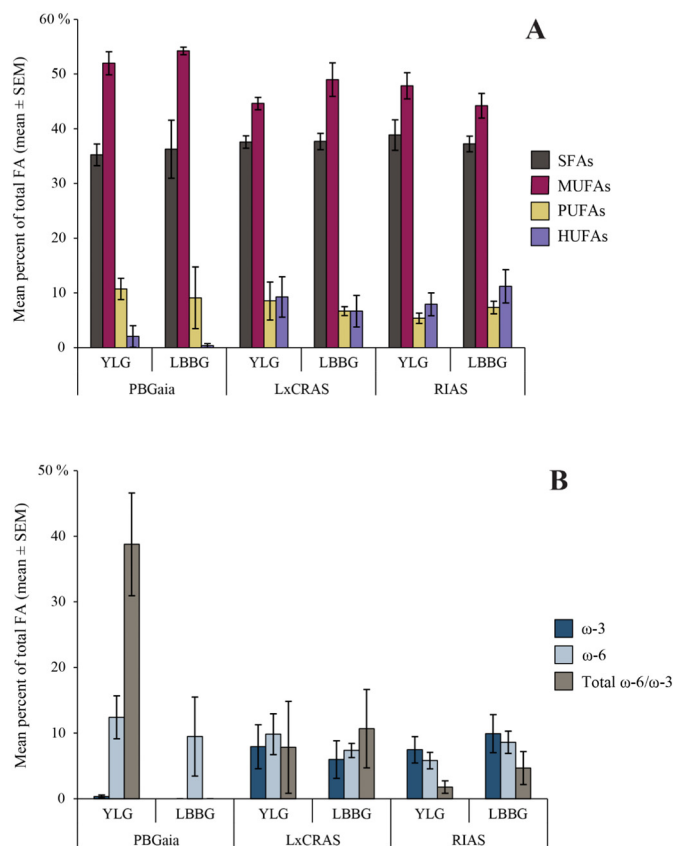


Fig. 2. Percentages of A) saturated (SFAs), monounsaturated (MUFAs), polyunsaturated (PUFAs) and highly unsaturated (HUFAs) fatty acids and B) omega (ω -3, ω -6 and total ω -6/ ω -3 fatty acids ratio in adipose tissue of yellow-legged (YLG, *Larus michahellis*) and lesser black-backed gulls (LBBG, *Larus fuscus*) from three wildlife rescue centres (PBGaia, LxCRAS and RIAS, where individuals from PBGaia represent the most urbanized gull population, gulls from RIAS represent the most natural individuals and LxCRAS individuals are both natural and urban dwellers). Data are plotted as means \pm standard error of the means (SEM).

remaining rescue centres (grey ellipse for LxCRAS and blue ellipse for RIAS in Fig. 3).

The PLS-DA loadings (Fig. 4) revealed that DHA (C22:6n-3), C17:0 and EPA (C20:5n-3 all higher in RIAS and LxCRAS) as well as C18:1n-9, LA (C18:2n-6) and C12:0 (all higher in PBGaia) were the FAs more important in explaining variation along comp1 and, therefore, in segregating wildlife rescue centres (Figs. 3 and 4A). C13:0 and C20:2n-6, negatively, as well as C20:0 and DGLA (C20:3n-6), positively, were the FAs more important in explaining variation along comp2 (Fig. 4B). The FAs

C15:0 and ARA (C20:4n-6) were the most important in explaining variation along comp3 (negatively and positively, respectively, Fig. 4C).

Visually, YLG and LBBG did not group distinctly in the respective PLS-DA and presented a high overlap between FA percentages (for ellipses of both species, see Fig. S1). None of the remaining gulls' characteristics (age, sex, BCS and clinical history) grouped distinctly in each corresponding PLS-DA (Fig. S2), presenting a high overlap between age classes, sexes, body condition scores and clinical histories.

3.3. Influence of the ingestion of anthropogenic materials on gulls' FA composition

From the 47 individuals studied, 25 (53.2%) had anthropogenic materials in their digestive systems with a mean (\pm SD) number of items of 2.13 ± 3.25 per individual (range 0–14 pieces), weighting 0.0703 ± 0.1633 g (range 0.0001–0.7867 g, Table 3). Detailed description of the anthropogenic materials found in gulls' digestive tract for each species and wildlife rescue centre, and the colours of the ingested materials can be found on Table S3 and Fig. S3, respectively.

No differences were detected in the number of items (all debris) found in gulls' digestive tract neither among rescue centres nor among species (Table 4). The number of plastic items was significantly higher for gulls from PBGaia than for gulls from LxCRAS ($Z = -2.26$; $p = 0.02$, Table 4). Mass of anthropogenic materials did not differ significantly among rescue centres, but LBBG had materials with greater mass in their digestive systems than YLG ($F_{1,23} = 6.26$; $p = 0.02$, Table 4).

YLG's body mass was not significantly related with the number of ingested items ($F_{1,21} = 0.59$, $p = 0.45$), but the number of ingested items was positively related with LBBG's body mass ($\beta = 9.75 \pm 4.37$ g number of items $^{-1}$, $r^2 = 0.18$, $F_{1,22} = 4.97$, $p = 0.04$, Table S4). However, there was no significant relationship between the mass of ingested anthropogenic materials and gulls' body mass ($F_{1,45} = 1.28$, $p = 0.26$), both in terms of wildlife rescue centre and gull species (Table S4). As for the number of ingested items, no significant relation was found with gulls' body mass neither considering all data ($F_{1,45} = 2.2$, $p = 0.15$), nor considering each wildlife rescue centre separately (Table S4).

The PLSR used to address the effect of the ingestion of anthropogenic materials on gulls' FA composition showed no clear pattern between the mass of ingested materials and FA composition (Fig. S4).

4. Discussion

In this study, the FA composition of two opportunistic gull species from three wildlife rescue centres representative of areas with different levels of urbanization was described and related with the ingestion of anthropogenic materials. We detected significant differences in gulls' FA composition between the three rescue centres, and therefore,

Table 2

Statistics from the A) general linear models (GLMs) testing the effect of wildlife rescue centre (PBGaia, LxCRAS and RIAS, where individuals from PBGaia represent the most urbanized gull population, gulls from RIAS represent the most natural individuals and LxCRAS individuals are both natural and urban) and species (yellow-legged gull, YLG, *Larus michahellis* and lesser black-backed gull, LBBG, *Larus fuscus*) on the percentages of each fatty acids (FA) group (SFAs, MUFAs, PUFAs, HUFAs, ω -3 and ω -6) and on the total ω -6/ ω -3 ratio from the adipose tissue of 47 gulls, and B) Tukey adjusted p -values of pairwise post-hoc comparisons among wildlife rescue centres. Significant effects are highlighted in bold. SFAs = saturated fatty acids; MUFAs = monounsaturated fatty acids; PUFAs = polyunsaturated fatty acids; HUFAs = highly unsaturated fatty acids.

FA group (mean %)	A		B			Main effect rescue centre
	Rescue centre	Species	PBGaia - LxCRAS	LxCRAS - RIAS	PBGaia - RIAS	
SFAs	$F_{2,44} = 0.8$; $p = 0.46$	$F_{1,45} = 0.03$; $p = 0.86$	0.575	0.988	0.453	
MUFAs	$F_{2,44} = 2.77$; $p = 0.07$	$F_{1,45} = 0.65$; $p = 0.42$	0.279	0.734	0.06	
PUFAs	$F_{2,44} = 3.23$; $p = \mathbf{0.05}$	$F_{1,45} = 0.62$; $p = 0.43$	0.143	0.889	0.045	RIAS > PBGaia
HUFAs	$F_{2,44} = 3.7$; $p = \mathbf{0.03}$	$F_{1,45} = 1.12$; $p = 0.3$	0.183	0.669	0.026	RIAS > PBGaia
ω -3	$F_{2,44} = 5.28$; $p = \mathbf{0.009}$	$F_{1,45} = 1.54$; $p = 0.22$	0.08	0.617	0.007	RIAS > PBGaia
ω -6	$F_{2,44} = 2.08$; $p = 0.14$	$F_{1,45} = 0.45$; $p = 0.51$	0.263	0.949	0.132	
Total ω -6/ ω -3	$F_{2,33} = 17.7$; $p < \mathbf{0.001}$	$F_{1,34} = 1.58$; $p = 0.22$	<0.001	0.332	<0.001	PBGaia > Others

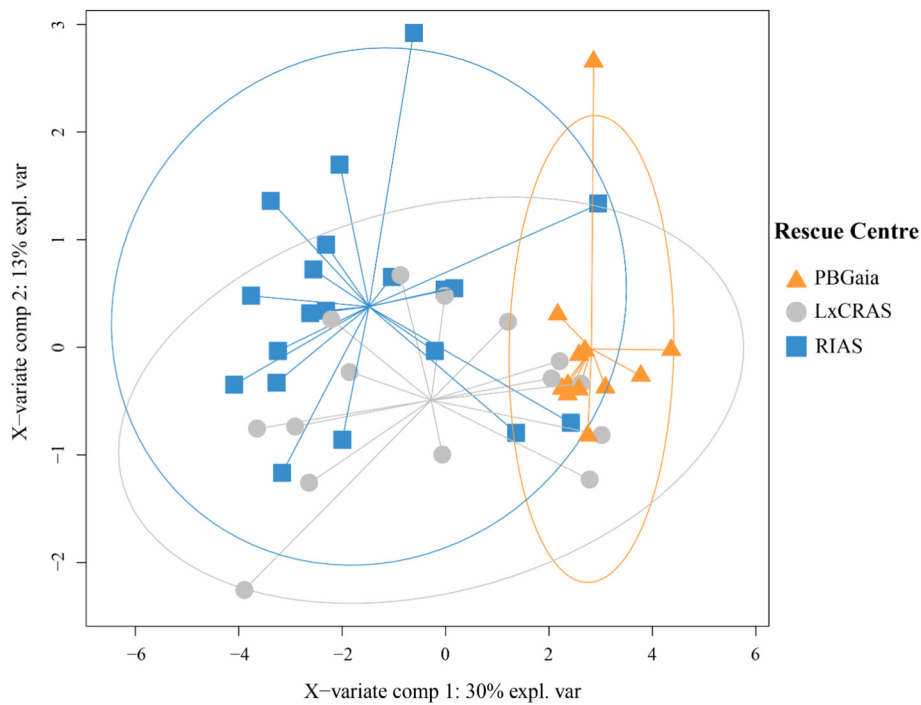


Fig. 3. Partial Least Squares Discriminant Analysis (PLS-DA) score plot (component 1 and component 2) of 47 gulls' adipose tissue fatty acids mean percentages (arcsine transformed) separated according to wildlife rescue centre (PBGaia: orange triangles; LxCRAS: grey points; RIAS: blue squares, where individuals from PBGaia represent the most urbanized gull population, gulls from RIAS represent the most natural individuals and LxCRAS individuals are both natural and urban dwellers). Each triangle, point or square represents each necropsied gull. 30% and 13% of the variance in fatty acids is explained by component 1 and component 2, respectively. Coloured ellipses represent 95% confidence intervals.

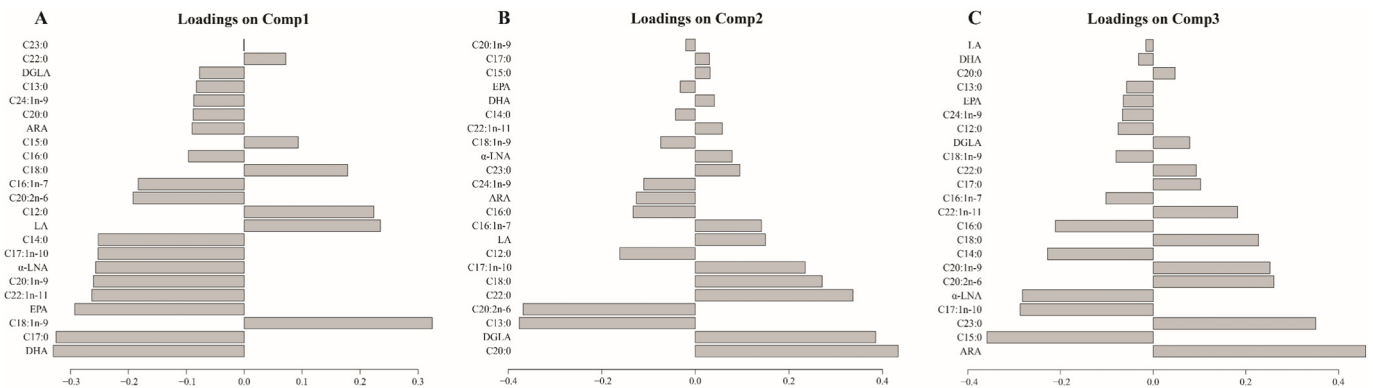


Fig. 4. Partial Least Squares Discriminant Analysis (PLS-DA) loadings plot of each one of the first three components (A: component 1, B: component 2 and C: component 3) of 47 gulls' adipose tissue fatty acids mean percentages (arcsine transformed) separated according to wildlife rescue centre. LA = linoleic acid (C18:2n-6); α LNA = α -linolenic acid (C18:3n-3); DGLA = dihomo- γ -linolenic acid (C20:3n-6); ARA = arachidonic acid (C20:4n-6); EPA = eicosapentaenoic acid (C20:5n-3); DHA = docosahexaenoic acid (C22:6n-3).

Table 3

Description of anthropogenic materials (debris) items present in 47 yellow-legged (YLG, *Larus michahellis*) and lesser black-backed gulls (LBBG *Larus fuscus*) necropsied at three wildlife rescue centres along Portugal (PBGaia, LxCRAS and RIAS, where individuals from PBGaia represent the most urbanized gull population, gulls from RIAS represent the most natural individuals and LxCRAS individuals are both natural and urban dwellers). FO = Frequency of Occurrence. SD = Standard Deviation. NA = Not Applicable.

Rescue centre	Species	No. individuals	FO (%) of debris	Items per individual		Mass of debris (g)		Size of debris (cm)		Total items
				Mean \pm SD	Range	Mean \pm SD	Range	Mean \pm SD	Range	
PBGaia	YLG	10	80	2.5 \pm 2.42	0–7	0.0108 \pm 0.0115	0.0001–0.0338	5.87 \pm 2.83	2.5–11	25
	LBBG	2	50	6 \pm 8.49	0–12	0.0880 \pm NA	0.0880–0.0880	16.5 \pm NA	16.5–16.5	12
LxCRAS	YLG	4	0							0
	LBBG	11	45.5	0.91 \pm 1.76	0–6	0.0362 \pm 0.0278	0.0055–0.0780	6.68 \pm 1.56	5–9	10
RIAS	YLG	9	55.6	2.22 \pm 3.15	0–9	0.0713 \pm 0.1358	0.0013–0.3132	4.34 \pm 2.5	2.9–8.78	20
	LBBG	11	54.6	3 \pm 4.2	0–14	0.1741 \pm 0.3043	0.0034–0.7867	10.62 \pm 6.45	4.75–23	33
PBGaia		12	75	3.08 \pm 3.63	0–12	0.0194 \pm 0.0279	0.0001–0.0880	7.05 \pm 4.42	2.5–16.5	37
LxCRAS		15	33.3	0.67 \pm 1.54	0–6	0.0362 \pm 0.0278	0.0055–0.0780	6.68 \pm 1.56	5–9	10
RIAS		20	55	2.65 \pm 3.69	0–14	0.1274 \pm 0.2378	0.0013–0.7867	7.76 \pm 5.83	2.9–23	53
YLG		23	56.5	1.96 \pm 2.62	0–9	0.0341 \pm 0.0846	0.0001–0.3132	5.28 \pm 2.71	2.5–11	45
LBBG		24	50	2.29 \pm 3.8	0–14	0.1095 \pm 0.2171	0.0034–0.7867	9.47 \pm 5.34	4.75–23	55
Total		47	53.2	2.13 \pm 3.25	0–14	0.0703 \pm 0.1633	0.0001–0.7867	7.29 \pm 4.61	2.5–23	100

Table 4

Statistics from A) zero-inflated models and B) general linear models testing the effect of wildlife rescue centre (PBGaia, LxCRAS and RIAS, where individuals from PBGaia represent the most urbanized gull population, gulls from RIAS represent the most natural individuals and LxCRAS individuals are both natural and urban dwellers) and species (YLG, *Larus michahellis* and LBBG, *Larus fuscus*), in the number (A) and mass (B) of anthropogenic materials (all debris) and in the number (A) and mass (B) of plastic items (all plastic) detected in 47 necropsied gulls. For zero-inflated models (A), PBGaia and LBBG were assigned as reference categories for rescue centre and species, respectively, and only results from count models are shown. Significant effects are highlighted in bold.

A		Rescue centre		Species		
No Items	All debris	LxCRAS	RIAS	Main effect	LBBG	Main effect
		$\beta \pm SE = -1.14 \pm 0.75$ $Z = -1.52$ $p = 0.13$	$\beta \pm SE = 0.2 \pm 0.5$ $Z = 0.40$ $p = 0.69$	-	$\beta \pm SE = -0.39 \pm 0.53$ $Z = -0.74$ $p = 0.46$	-
	All plastic	$\beta \pm SE = -2.61 \pm 1.16$ $Z = -2.26$ $p = \mathbf{0.02}$	$\beta \pm SE = -0.35 \pm 0.83$ $Z = -0.43$ $p = 0.67$	PBGaia > Others	$\beta \pm SE = -0.78 \pm 0.84$ $Z = -0.93$ $p = 0.35$	-
B		Rescue Centre		Species		
Mass	All Debris	$F_{2,22} = 1.32; p = 0.29$	-	$F_{1,23} = 6.26; p = \mathbf{0.02}$	LBBG > YLG	
	All Plastic	$F_{2,11} = 0.30; p = 0.75$	-	$F_{1,12} = 1.71; p = 0.22$	-	

among urbanization levels, but not among species. A significant positive relation between the number of ingested items and LBBG's body mass was detected, but we were unable to identify any effect of the mass of ingested anthropogenic materials on gulls' FA composition.

4.1. Differences in FA composition

Gulls from the rescue centre that represents the most urbanized area (PBGaia) had significantly lower percentages of physiologically important groups of FAs (HUFAs, PUFAs and ω -3 FAs) in their adipose tissue than gulls from the remaining centres (LxCRAS and RIAS). We identified the FAs responsible for the segregation between rescue centres, and therefore, important in separating highly urbanized areas from more natural habitats. The FAs DHA, C17:0 and EPA presented significantly higher percentages in RIAS and LxCRAS, which are representative of more natural habitats, whereas C18:1n-9, LA and C12:0 were higher in PBGaia, the most urbanized location.

Gulls adipose tissue revealed a clear predominance of MUFAs rather than SFAs. This is in accordance with other studies that report, for instance, higher amounts of C18:1n-9 (MUFA) than C18:0 (SFA) on seabirds' fat tissue (Dahl et al., 2003; K  kel   et al., 2006; Puskic et al., 2019), which is often related with a diet enriched on marine species (Dahl et al., 2003). However, individuals from PBGaia (the most urbanized location) exhibited particularly higher percentages of the FA C18:1n-9, accounting for 44% of the total FAs for that rescue centre vs. 28% in RIAS (the least urbanized site) and 34% in LxCRAS. Urban gulls from PBGaia should have a highly diverse diet, including the presence of anthropogenic food items in their diets such as remnants of human meals, as reported for other gulls using and relying on urban habitats (Egunez et al., 2018; Huig et al., 2016; Pais de Faria et al., 2021b; Real et al., 2017). Unfavourable physiological states characterized by loss of body mass or periodic fasting associated with breeding, moult or migration, which can be enhanced by a nutritionally poorer diet in urban habitats, may result in the selective mobilization of certain FAs, and *de novo* biosynthesis of other FAs like C16:0 and C18:0, as well as their respective products C16:1n-7 and C18:1n-9 (Williams and Buck, 2010). This may explain the higher proportions of C18:1n-9 in individuals from PBGaia. Still, the major factor affecting FA composition is diet and, although in smaller amounts, SFAs and MUFAs are also obtained from diet (Iverson et al., 2007). Despite the highly diverse diet reported for urban gulls from Porto, they still relied on marine resources throughout the year (Pais de Faria et al., 2021b), which also may help in understanding the higher C18:1n-9 proportions in PBGaia urban gulls.

The SFA palmitic acid (C16:0) was the second most common FA on adipose tissue of the studied individuals, in similar proportions for each species and per wildlife rescue centre. This SFA, along with the stearic acid (C18:0), are two of the most abundant FAs found in animals,

and is a common released product from the *de novo* synthesis pathway of 14-carbon FAs within seabirds' liver (Dalsgaard et al., 2003; K  kel   et al., 2009). This FA may be stored in the adipose tissue or used rapidly as an energy substrate (Williams and Buck, 2010). Palmitic acid was the most abundant FA found in plasma of YLG fledglings in Porto urban breeding colony and in Berlenga natural breeding colony (Pais de Faria et al., 2021b). By being biosynthesised *de novo* by birds, both SFAs and MUFAs relative levels can be controlled to a larger extent than the levels of PUFAs and HUFAs (Isaksson et al., 2017), therefore these SFAs and MUFAs results are likely a consequence of metabolic regulation combined with habitat specific diet.

Essential fatty acids (EFAs), such as the ω -3 EPA and DHA cannot be synthesised *de novo* and must be obtained through diet, being extremely important to bird physiology (Dalsgaard et al., 2003; Gladyshev et al., 2009). In fact, EPA and DHA, both ω -3 FAs, were the most important FAs in segregating wildlife rescue centres, all showing higher percentages in individuals from RIAS, the least urbanized area, and LxCRAS. The higher percentages of these ω -3 FAs in individuals from LxCRAS and RIAS is consistent with a diet based on marine resources (Calado et al., 2018, 2021; Dalsgaard et al., 2003). On the contrary, the deficiency in ω -3 FAs and the lower diversity of FAs (i.e. mean number of FAs per individual, Table 1) in gulls from the most urbanized location (PBGaia) are indicators of terrestrial food-webs (Taipale et al., 2014; Twining et al., 2018), suggesting a diet based on anthropogenic food resources. This FA composition suggests that urban dwellers from PBGaia, have a poorer nutritional condition as items typically found in human meal leftovers are usually rich in fat and proteins, allowing a greater energy intake, but might be deficient in essential nutrients (Patenaude-Monette et al., 2014). The ω -3 FAs deficiency in individuals from PBGaia is in accordance with previous work performed with gull fledglings from the urban colony of Porto (Pais de Faria et al., 2021b). The higher ω -6: ω -3 FAs ratio of individuals from PBGaia may be suggestive of a higher propensity by urban gulls to an enhanced diet-induced susceptibility to inflammation when exposed to antigens, and to suffer from a higher oxidative stress status (Isaksson, 2015; Isaksson et al., 2017; Romieu et al., 2008). Yet, we cannot exclude the possibility that the levels of FAs detected by this study could be within the range of healthy and normal FA variability and, therefore, may not translate into health problems. An urban diet, typically rich in anthropogenic food resources and poor in marine items, and consequently with low levels of ω -3 EFAs, may be responsible for lower egg quality and reduced chick weight in urban gulls (Dosch, 1997; Hebert et al., 2020).

None of the gulls' characteristics (species, age, sex, body condition score, clinical history) seemed to be important in explaining the global variation in FA composition. Auman et al. (2008) described sex-based differences in condition of urban gulls: males were heavier and larger than the urban females of silver gull (*Larus novaehollandiae*). Such

variation in condition could be further reflected in FA differences between males and females, as reported by Käkälä et al. (2006) for great skuas (*Stercorarius skua*). The great skuas' FA variations were attributed to sexual size dimorphism and division of labour while breeding. In YLG and LBBG species, both male and female share their nest and chick duties, and both leave the nest for feeding themselves and to provision the chicks. Still, gull males are typically larger than females (Arizaga et al., 2008) which could be responsible for sex-differences in FA composition. Both YLG and LBBG, adults and immatures, males and females, are known to benefit from reliable and predictable food sources, either by interacting with fishing boats and feeding on marine species with higher nutritional value (i.e. fishery discards; Calado et al., 2018, 2021; Mendes et al., 2018), mainly in natural habitats, or by feeding on human meal leftovers collected from trash containers or in nearby landfills, in urban habitats (Lopes et al., 2021; Pais de Faria et al., 2021a; Spelt et al., 2019). Also, in this study, we compared FA composition between adults and immature gulls (1–3 years), and we did not consider fledglings. All gulls were captured during their non-breeding season (September to January) and adipose tissue reflects a diet integrated over a period of 1–2 months (Williams and Buck, 2010), therefore adults and immatures of both sexes could be experiencing a similar energy-demanding status as gulls were not breeding.

We acknowledge that some individuals in each habitat may not be strict urban or natural dwellers as we stated, because the movement ecology of each individual before capture is unknown. However, our previous research indicates that gulls from Porto are largely urban dwellers year-round (Pais de Faria et al., 2021a, 2021b), and those from Ria Formosa forage mostly in interaction with fishing activities also year-round (Calado et al., 2021).

When comparing to pellet and bolus analysis, the use of necropsies presents several advantages including the possibility of determining age, sex, health status and cause of death of the individuals, evaluating the entire burden of anthropogenic materials, assessing potential internal pathologies (i.e. macroscopic lesions and related pathological patterns) and sampling internal tissues for subsequent histopathological or chemical analysis (Provencher et al., 2019). The use of animals from wildlife rehabilitation centres may have skewed our samples as these individuals were likely in a poorer health condition, presenting altered physiological conditions beforehand that may have been confounded with the treatment effects and introduced bias to our results. Despite being an opportunistic methodology in relation to season or species, it allows repeated sampling and constitutes a non-invasive approach, as individuals are not purposely collected or killed for scientific research, with the collection of a large amount of data on each individual (Provencher et al., 2017).

4.2. Debris ingestion and FA composition

Overall, our study detected that 53.2% and 29.8% of the 47 necropsied gulls had anthropogenic materials and plastics, respectively, in their digestive systems, with a mean of 2.13 debris items per individual and a mean of 0.77 plastic items per individual. These values are relatively similar to those of other gull debris studies using necropsies (review by Seif et al., 2018). In previous studies, both YLG and LBBG exhibited high levels of anthropogenic materials in their pellets (Alonso et al., 2015; Calado et al., 2018), especially in urban and landfill environments (Lopes et al., 2021). However, the use of necropsies only allows for the detection of a smaller amount of debris in gulls' digestive system (Basto et al., 2019; Codina-García et al., 2013; this study) since gulls have the ability to regurgitate a large part of non-edible food remnants, including anthropogenic materials (Barrett et al., 2007), reducing the time that these materials are in individuals' digestive system. In fact, previous pellet analysis from breeding gulls of the same study areas indicate a large amount of regurgitated anthropogenic materials, particularly plastics by the urban gulls of Porto (Lopes et al., 2021). This regurgitation capability allows the rejection of larger and heavier

items that were more likely to physically block and/or damage gulls' digestive tract and, therefore, items with greater masses and sizes may have a lower impact on their digestive system. Still, some items are known to remain in the gulls' digestive tracts (i.e. smaller items and microplastics which may not be regurgitated, Provencher et al., 2017), with an unknown retention time, and these may be more likely to interfere with birds' physiology and body condition (Puskic et al., 2019), but comprehensive studies on how these debris items affect birds' health are scarce, especially in an urbanization context.

Body mass of fledgling flesh-footed shearwaters (*Ardenna carneipes*) was inversely proportional to the mass and the number of ingested plastic items, which may indicate sub-lethal effects of plastic pollution on marine wildlife (Lavers et al., 2014). However, with a similar analysis, Puskic et al. (2019) failed to detect a relationship between ingested plastics, linear morphometrics and FA composition. Determining body condition as body mass corrected for size may not be the best metric to detect effects on animals which have ingested plastic and other anthropogenic materials, hence the reason why FA analysis are being applied to explore such problem. We were not able to detect a relation between the ingestion of anthropogenic materials and FA composition, and this may have different explanations. In fact, beyond the ingestion of debris materials, other factors may be influencing our results. First, by choosing individuals with similar symptoms and identical degrees of disease (i.e. paretic syndrome), we attempted to reduce variability regarding their health status and clinical history, still we can not exclude the possibility of bias in our samples that affected FA profile other than the ingestion of anthropogenic materials. Second, the amount of anthropogenic materials in gulls' guts turned out to be quite low comparing to what we were expecting, especially for urban gulls. Although gulls are known to ingest large amounts of anthropogenic materials, such debris may have been "excreted" via the production of pellets (see Lopes et al., 2021) and, therefore, such levels of ingested anthropogenic materials may simply be below toxic levels and may not cause impairment nor sub-lethal impacts on the studied individuals. This is also a reminder of the seasonal variability in debris ingestion, the individuals' responses to ingestion, and ultimately the difficulty of identifying sub-lethal impacts of the ingestion of anthropogenic materials in seabirds (Rochman et al., 2016; Roman et al., 2019).

In conclusion, gulls inhabiting urban habitats may have some immediate benefits when compared to gulls living in natural habitats, such as reduced foraging energetic costs due to the high availability and accessibility of anthropogenic food resources. Our study suggests that FA composition of urban gulls has lower nutritional quality than that of gulls inhabiting more natural habitats, and such nutritional costs may have long-term effects for urban dwelling populations which deserve further studies. FA analysis is thus a useful tool to elucidate how anthropogenic materials may disturb metabolic pathways and to assess the less visible impacts of their ingestion, even though our results suggest that, at least with our sample of birds from a small period of time, there was no such effect. In the long run, urban gulls may be more exposed to several contaminants, pathogens (Alm et al., 2018; Sorais et al., 2020) and anthropogenic materials (Lopes et al., 2020, 2021) that might endanger gulls' health condition, survival and/or reproductive output.

Ethical approval

All experiments and protocols performed in this study comply with current Portuguese legislation.

CRediT authorship contribution statement

Catarina S. Lopes: Conceptualization, Investigation, Funding acquisition, Writing – original draft, Writing – review & editing. **Raquel C.C. Antunes:** Investigation, Writing – review & editing. **Vitor H. Paiva:** Supervision, Funding acquisition, Writing – review & editing. **Ana M.M.**

Gonçalves: Supervision, Funding acquisition, Methodology, Writing – review & editing. **Jorge J. Correia:** Supervision, Writing – review & editing. **Jaime A. Ramos:** Supervision, Funding acquisition, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

We are thankful to all the wildlife rescue centres and personnel that provided samples and logistic support to perform necropsies, namely Dr. Vanessa Soeiro (PBGaia), Dr. Maria Manuela Mira (LxCRAS), Dr. Erica Brazio (LxCRAS) and Dr. Maria Casero (RIAS).

We acknowledge the support of Portuguese national funds provided by the Foundation for Science and Technology, I.P. (FCT), within the scope of the projects UIDB/04292/2020 granted to MARE – Marine and Environmental Sciences Centre, UIDP/50017/2020+UIDB/50017/2020 (by FCT/MTCES) granted to CESAM – Centre for Environmental and Marine Studies, and to the project PORBIOTA – E-Infrastructure Portuguese Information and Research in Biodiversity (POCI-01-0145-FEDER-022127), supported by Competitiveness and Internationalization Operational Programme and Regional Operational Programme of Lisbon, through FEDER, FCT and national funds (OE). Catarina S. Lopes thanks FCT for the fellowship SFRH/BD/118862/2016. Ana M. M. Gonçalves acknowledges University of Coimbra for the contract IT057-18-7253.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.scitotenv.2021.151093>.

References

- Alm, E.W., Daniels-Witt, Q.R., Learman, D.R., Ryu, H., Jordan, D.W., Gehring, T.M., Santo Domingo, J., 2018. Potential for gulls to transport bacteria from human waste sites to beaches. *Sci. Total Environ.* 615, 123–130. <https://doi.org/10.1016/j.scitotenv.2017.09.232>.
- Alonso, H., Almeida, A., Granadeiro, J.P., Catty, P., 2015. Temporal and age-related dietary variations in a large population of yellow-legged gulls *Larus michahellis*: implications for management and conservation. *Eur. J. Wildl. Res.* 61, 819–829. <https://doi.org/10.1007/s10344-015-0958-9>.
- Andersson, M.N., Wang, H.-L., Nord, A., Salmón, P., Isaksson, C., 2015. Composition of physiologically important fatty acids in great tits differs between urban and rural populations on a seasonal basis. *Front. Ecol. Evol.* 3, 1–13. <https://doi.org/10.3389/fevo.2015.00093>.
- Annett, C.A., Pierotti, R., 1999. Long-term reproductive output in western gulls: consequences of alternate tactics in diet choice. *Ecology* 80, 288–297. [https://doi.org/10.1890/0012-9658\(1999\)080\[0288:LTR0IW\]2.0.CO;2](https://doi.org/10.1890/0012-9658(1999)080[0288:LTR0IW]2.0.CO;2).
- Arizaga, J., Aldalur, A., Herrero, A., Galicia, D., 2008. Sex differentiation of yellow-legged gull (*Larus michahellis lusitanicus*): the use of biometrics, bill morphometrics and wing tip coloration. *Waterbirds* 31, 211–219. [https://doi.org/10.1675/1524-4695\(2008\)31\[211:SDOYGL\]2.0.CO;2](https://doi.org/10.1675/1524-4695(2008)31[211:SDOYGL]2.0.CO;2).
- Aronson, M.F.J., La Sorte, F.A., Nilon, C.H., Katti, M., Goddard, M.A., Lepczyk, C.A., Warren, P.S., Williams, S.G., Cilliers, S., Clarkson, B., Dobbs, C., Dolan, R., Hedblom, M., Klotz, S., Kooijmans, J.L., Siebert, S., Sushinsky, J., Werner, P., Winter, M., 2014. A global analysis of the impacts of urbanization on bird and plant diversity reveals key anthropogenic drivers. *Proc. R. Soc. B Biol. Sci.* 281.
- Auman, H.J., Ludwig, J.P., Giesy, J.P., Colborn, T., 1997. Plastic ingestion by Laysan albatross chicks on Sand Island, Midway Atoll, in 1994 and 1995. In: Robinson, G., Gales, R. (Eds.), *Albatross Biology and Conservation*. Chipping Norton, pp. 239–244.
- Auman, H.J., Meathrel, C.E., Richardson, A., 2008. Superize me: does anthropogenic food change the body condition of silver Gulls? A comparison between urbanized and remote, non-urbanized areas. *Waterbirds* 31, 122–126. [https://doi.org/10.1675/1524-4695\(2008\)31\[122:SMDAFC\]2.0.CO;2](https://doi.org/10.1675/1524-4695(2008)31[122:SMDAFC]2.0.CO;2).
- Barrett, R.T., Camphuysen, K.C.J., Anker-Nilssen, T., Chardine, J.W., Furness, R.W., Garthe, S., Hüppop, O., Leopold, M.F., Montevecchi, W.A., Veit, R.R., 2007. Diet studies of seabirds: a review and recommendations. *ICES J. Mar. Sci.* 64, 1675–1691. <https://doi.org/10.1093/icesjms/fsm152>.
- Bartumeus, F., Giuggioli, L., Louzao, M., Bretagnolle, V., Oro, D., Levin, S.A., 2010. Fishery discards impact on seabird movement patterns at regional scales. *Curr. Biol.* 20, 215–222. <https://doi.org/10.1016/j.cub.2009.11.073>.
- Basto, M.N., Nicastro, K.R., Tavares, A.I., McQuaid, C.D., Casero, M., Azevedo, F., Zardi, G.L., 2019. Plastic ingestion in aquatic birds in Portugal. *Mar. Pollut. Bull.* 138, 19–24. <https://doi.org/10.1016/j.marpolbul.2018.11.024>.
- Battisti, C., Staffieri, E., Poeta, G., Sorace, A., Luiselli, L., Amori, G., 2019. Interactions between anthropogenic litter and birds: a global review with a ‘black-list’ of species. *Mar. Pollut. Bull.* 138, 93–114. <https://doi.org/10.1016/j.marpolbul.2018.11.017>.
- Belant, J.L., 1997. Gulls in urban environments: landscape-level management to reduce conflict. *Landsc. Urban Plan.* 38, 245–258. [https://doi.org/10.1016/S0169-2046\(97\)00037-6](https://doi.org/10.1016/S0169-2046(97)00037-6).
- Bouland, A.J., White, A.E., Lonabaugh, K.P., Varian-Ramos, C.W., Cristol, D.A., 2012. Female-biased offspring sex ratios in birds at a mercury-contaminated river. *J. Avian Biol.* 43, 244–251. <https://doi.org/10.1111/j.1600-048X.2012.05612.x>.
- Calado, J.G., Matos, D.M., Ramos, J.A., Moniz, F., Ceia, F.R., Granadeiro, J.P., Paiva, V.H., 2018. Seasonal and annual differences in the foraging ecology of two gull species breeding in sympatry and their use of fishery discards. *J. Avian Biol.* 49, 1–12. <https://doi.org/10.1111/jav.01463>.
- Calado, J.G., Veríssimo, S.N., Paiva, V.H., Ramos, R., Vaz, P.T., Matos, D.M., Pereira, J., Lopes, C.S., Oliveira, N., Quaresma, A., Ceia, F.R., Velando, A., Ramos, J.A., 2021. Influence of fisheries on the spatio-temporal feeding ecology of gulls along the western iberian coast. *Mar. Ecol. Prog. Ser.* 661, 187–201. <https://doi.org/10.3354/meps13601>.
- Ceia, F.R., Patrício, J., Marques, J.C., Dias, J.A., 2010. Coastal vulnerability in barrier islands: the high-risk areas of the ria Formosa (Portugal) system. *Ocean Coast. Manag.* 53, 478–486. <https://doi.org/10.1016/j.ocecoaman.2010.06.004>.
- Ceia, F.R., Paiva, V.H., Fidalgo, V., Morais, L., Baeta, A., Crisóstomo, P., Mourato, E., Garthe, S., Marques, J.C., Ramos, J.A., 2014. Annual and seasonal consistency in the feeding ecology of an opportunistic species, the yellow-legged gull *Larus michahellis*. *Mar. Ecol. Prog. Ser.* 497, 273–284. <https://doi.org/10.3354/meps10586>.
- Codina-García, M., Militão, T., Moreno, J., González-Solis, J., 2013. Plastic debris in Mediterranean seabirds. *Mar. Pollut. Bull.* 77, 220–226. <https://doi.org/10.1016/j.marpolbul.2013.10.002>.
- Connors, P.G., Smith, K.G., 1982. Oceanic plastic particle pollution: suspected effect on fat deposition in red phalaropes. *Mar. Pollut. Bull.* 13, 18–20. [https://doi.org/10.1016/0025-326X\(82\)90490-8](https://doi.org/10.1016/0025-326X(82)90490-8).
- Costa, R.A., Sá, S., Pereira, A.T., Ferreira, M., Vingada, J.V., Eira, C., 2021. Threats to seabirds in Portugal: integrating data from a rehabilitation Centre and stranding network. *Eur. J. Wildl. Res.* 67. <https://doi.org/10.1007/s10344-021-01483-5>.
- Dahl, T.M., Falk-Petersen, S., Gabrielsen, G.W., Sargent, J.R., Hop, H., Millar, R.M., 2003. Lipids and stable isotopes in common eider, black-legged kittiwake and northern fulmar: atrophic study from an Arctic fjord. *Mar. Ecol. Prog. Ser.* 256, 257–269. <https://doi.org/10.3354/meps256257>.
- Dalsgaard, J., St. John, M., Kattner, G., Müller-Navarra, D., Hagen, W., 2003. Fatty Acid Trophic Markers in the Pelagic Marine Environment, pp. 225–340 [https://doi.org/10.1016/S0065-2881\(03\)46005-7](https://doi.org/10.1016/S0065-2881(03)46005-7).
- Dosch, J.J., 1997. Diet of nestling laughing gulls in southern New Jersey. *Waterbirds* 20, 273–281. <https://doi.org/10.2307/1521693>.
- Duhem, C., Roche, P., Vidal, E., Taton, T., 2008. Effects of anthropogenic food resources on yellow-legged gull colony size on Mediterranean islands. *Popul. Ecol.* 50, 91–100. <https://doi.org/10.1007/s10144-007-0059-z>.
- Egunez, A., Zorrozu, N., Aldalur, A., Herrero, A., Arizaga, J., 2018. Local use of landfills by a yellow-legged gull population suggests distance-dependent resource exploitation. *J. Avian Biol.* 49, jav-01455. <https://doi.org/10.1111/jav.01455>.
- Fuirst, M., Veit, R.R., Hahn, M., Dheilly, N., Thorne, L.H., 2018. Effects of urbanization on the foraging ecology and microbiota of the generalist seabird *Larus argentatus*. *PLoS One* 13, 1–22. <https://doi.org/10.1371/journal.pone.0209200>.
- Gallo, F., Fossi, C., Weber, R., Santillo, D., Sousa, J., Ingram, I., Nadal, A., Romano, D., 2018. Marine litter plastics and microplastics and their toxic chemicals components: the need for urgent preventive measures. *Environ. Sci. Eur.* 30. <https://doi.org/10.1186/s12302-018-0139-z>.
- Gladyshev, M.I., Arts, M.T., Sushchik, N.N., 2009. Preliminary estimates of the export of omega-3 highly unsaturated fatty acids (EPA+DHA) from aquatic to terrestrial ecosystems. In: Kainz, M.J., Brett, M.T., Arts, M.T. (Eds.), *Lipids in Aquatic Ecosystems*. Springer New York, New York, NY, pp. 179–210 https://doi.org/10.1007/978-0-387-89366-2_8.
- Gonçalves, A.M.M., Azeiteiro, U.M., Pardal, M.A., De Troch, M., 2012. Fatty acid profiling reveals seasonal and spatial shifts in zooplankton diet in a temperate estuary. *Estuar. Coast. Shelf Sci.* 109, 70–80. <https://doi.org/10.1016/j.jecss.2012.05.020>.
- Gregory, M.R., 2009. Environmental implications of plastic debris in marine settings - entanglement, ingestion, smothering, hangers-on, hitch-hiking and alien invasions. *Philos. Trans. R. Soc. B Biol. Sci.* 364, 2013–2025. <https://doi.org/10.1098/rstb.2008.0265>.
- Gyimesi, A., Boudewijn, T.J., Buijs, R.-J., Shamoun-Baranes, J.Z., de Jong, J.W., Fijn, R.C., van Horssen, P.W., Poot, M.J.M., 2016. Lesser black-backed gulls *Larus fuscus* thriving on a non-marine diet. *Bird Study* 63, 241–249. <https://doi.org/10.1080/00063657.2016.1180341>.
- Hadi, A.S., Ling, R.F., 1998. Some cautionary notes on the use of principal components regression. *Am. Stat.* <https://doi.org/10.1080/00031305.1998.10480530>.
- Hebert, C.E., Weseloh, D.V.C., Arts, M.T., de Solla, S.R., Moore, D.J., Paterson, G., Pekarik, C., 2020. Trends in herring gull egg quality over four decades reflect ecosystem state. *J. Great Lakes Res.* 46, 538–548. <https://doi.org/10.1016/j.jglr.2020.03.004>.
- Henry, P.-Y., Wey, G., Balança, G., 2011. Rubber band ingestion by a rubbish dump dweller, the white stork (*Ciconia ciconia*). *Waterbirds* 34, 504–508. <https://doi.org/10.1675/063.034.0414>.

- Herzke, D., Anker-Nilssen, T., Nøst, T.H., Götsch, A., Christensen-Dalsgaard, S., Langset, M., Fangel, K., Koelmans, A.A., 2016. Negligible impact of ingested microplastics on tissue concentrations of persistent organic pollutants in northern fulmars off coastal Norway. *Environ. Sci. Technol.* 50, 1924–1933. <https://doi.org/10.1021/acs.est.5b04663>.
- Huig, N., Buijs, R.-J., Kleyheeg, E., 2016. Summer in the city: behaviour of large gulls visiting an urban area during the breeding season. *Bird Study* 63, 214–222. <https://doi.org/10.1080/00063657.2016.1159179>.
- Isaksson, C., 2015. Urbanization, oxidative stress and inflammation: a question of evolving, acclimatizing or coping with urban environmental stress. *Funct. Ecol.* 29, 913–923. <https://doi.org/10.1111/1365-2435.12477>.
- Isaksson, C., Hanson, M.A., Burdge, G.C., 2015. The effects of spatial and temporal ecological variation on fatty acid compositions of wild great tits *Parus major*. *J. Avian Biol.* 46, 245–253. <https://doi.org/10.1111/jav.00409>.
- Isaksson, C., Andersson, M.N., Nord, A., von Post, M., Wang, H.-L., 2017. Species-dependent effects of the urban environment on fatty acid composition and oxidative stress in birds. *Front. Ecol. Evol.* 5, 1–13. <https://doi.org/10.3389/fevo.2017.00044>.
- Iverson, S.J., Springer, A.M., Kitaysky, A.S., 2007. Seabirds as indicators of food web structure and ecosystem variability: qualitative and quantitative diet analyses using fatty acids. *Mar. Ecol. Prog. Ser.* 352, 235–244. <https://doi.org/10.3354/meps07073>.
- Jackman, S., 2017. *Pscl*: classes and methods for R developed in the political science computational laboratory. United States Studies Centre, University of Sydney. Sydney, New South Wales, Australia. R package version 1.5.2. <https://github.com/atahk/pscl/>.
- Käkelä, A., Crane, J., Votier, S.C., Furness, R.W., Käkelä, R., 2006. Fatty acid signatures as indicators of diet in great skuas *Stercorarius skua*, Shetland. *Mar. Ecol. Prog. Ser.* 319, 297–310. <https://doi.org/10.3354/meps319297>.
- Käkelä, R., Furness, R.W., Kahle, S., Becker, P.H., Käkelä, A., 2009. Fatty acid signatures in seabird plasma are a complex function of diet composition: a captive feeding trial with herring gulls. *Funct. Ecol.* 23, 141–149. <https://doi.org/10.1111/j.1365-2435.2008.01475.x>.
- Karnovskiy, N.J., Hobson, K.A., Iverson, S.J., 2012. From lavage to lipids: estimating diets of seabirds. *Mar. Ecol. Prog. Ser.* 451, 263–284. <https://doi.org/10.3354/meps09713>.
- Koelmans, A.A., Besseling, E., Foekema, E., Kooi, M., Mintenig, S., Ossendorp, B.C., Redondo-Hasselhar, P.E., Verschoor, A., Van Wezel, A.P., Scheffer, M., 2017. Risks of plastic debris: unravelling fact, opinion, perception, and belief. *Environ. Sci. Technol.* 51, 11513–11519. <https://doi.org/10.1021/acs.est.7b02219>.
- Kühn, S., van Franeker, J.A., 2020. Quantitative overview of marine debris ingested by marine megafauna. *Mar. Pollut. Bull.* 151, 110858. <https://doi.org/10.1016/j.marpolbul.2019.110858>.
- Kühn, S., Bravo Rebolledo, E.L., van Franeker, J.A., 2015. Deleterious effects of litter on marine life. In: Bergmann, M., Gutow, L., Klages, M. (Eds.), *Marine Anthropogenic Litter*. Springer International Publishing, Cham, pp. 75–116 https://doi.org/10.1007/978-3-319-16510-3_4.
- Lavers, J.L., Bond, A.L., 2016. Ingested plastic as a route for trace metals in laysan albatross (*Phoebastria immutabilis*) and bonin petrel (*Pterodroma hypoleuca*) from midway atoll. *Mar. Pollut. Bull.* 110, 493–500. <https://doi.org/10.1016/j.marpolbul.2016.06.001>.
- Lavers, J.L., Bond, A.L., Hutton, I., 2014. Plastic ingestion by flesh-footed shearwaters (*Puffinus carneipes*): implications for fledging body condition and the accumulation of plastic-derived chemicals. *Environ. Pollut.* 187, 124–129. <https://doi.org/10.1016/j.envpol.2013.12.020>.
- Lavers, J.L., Hutton, I., Bond, A.L., 2019. Clinical pathology of plastic ingestion in marine birds and relationships with blood chemistry. *Environ. Sci. Technol.* 53, 9224–9231. <https://doi.org/10.1021/acs.est.9b02098>.
- Lenth, R.V., 2016. Least-squares means: the R package lsmmeans. *J. Stat. Softw.* 69. <https://doi.org/10.18637/jss.v069.i01>.
- Lopes, C.S., Pais de Faria, J., Paiva, V.H., Ramos, J.A., 2020. Characterization of anthropogenic materials on yellow-legged gull (*Larus michahellis*) nests breeding in natural and urban sites along the coast of Portugal. *Environ. Sci. Pollut. Res.* 27, 36954–36969. <https://doi.org/10.1007/s11356-020-09651-x>.
- Lopes, C.S., Paiva, V.H., Vaz, P.T., Pais de Faria, J., Calado, J.G., Pereira, J.M., Ramos, J.A., 2021. Ingestion of anthropogenic materials by yellow-legged gulls (*Larus michahellis*) in natural, urban, and landfill sites along Portugal in relation to diet composition. *Environ. Sci. Pollut. Res.* <https://doi.org/10.1007/s11356-020-12161-5>.
- Marzluff, J.M., 2001. Worldwide urbanization and its effects on birds. *Avian Ecology and Conservation in an Urbanizing World*. Springer, pp. 19–47 https://doi.org/10.1007/978-1-4615-1531-9_2.
- Matos, D.M., Ramos, J.A., Calado, J.G., Ceia, F.R., Hey, J., Paiva, V.H., 2018. How fishing intensity affects the spatial and trophic ecology of two gull species breeding in sympatry. *ICES J. Mar. Sci.* 75, 1949–1964. <https://doi.org/10.1093/icesjms/fsy096>.
- Mendes, R.F., Ramos, J.A., Paiva, V.H., Calado, J.G., Matos, D.M., Ceia, F.R., 2018. Foraging strategies of a generalist seabird species, the yellow-legged gull, from GPS tracking and stable isotope analyses. *Mar. Biol.* <https://doi.org/10.1007/s00227-018-3421-0>.
- Méndez, A., Montalvo, T., Aymí, R., Carmona, M., Figuerola, J., Navarro, J., 2020. Adapting to urban ecosystems: unravelling the foraging ecology of an opportunistic predator living in cities. *Urban Ecosyst.* <https://doi.org/10.1007/s11252-020-00995-3>.
- Nager, R.G., O'Hanlon, N.J., 2016. Changing numbers of three gull species in the British Isles. *Waterbirds* 39, 15–28. <https://doi.org/10.1675/063.039.sp108>.
- Oro, D., Genovart, M., Tavecchia, G., Fowler, M.S., Martínez-Abraín, A., 2013. Ecological and evolutionary implications of food subsidies from humans. *Ecol. Lett.* 16, 1501–1514. <https://doi.org/10.1111/ele.12187>.
- Pais de Faria, J., Paiva, V.H., Veríssimo, S.N., Gonçalves, A.M.M., Ramos, J.A., 2021a. Seasonal variation in habitat use, daily routines and interactions with humans by urban-dwelling gulls. *Urban Ecosyst.* <https://doi.org/10.1007/s11252-021-01101-x>.
- Pais de Faria, J., Vaz, P.T., Lopes, C.S., Calado, J.G., Pereira, J.M., Veríssimo, S.N., Paiva, V.H., Gonçalves, A.M.M., Ramos, J.A., 2021b. The importance of marine resources in the diet of urban gulls. *Mar. Ecol. Prog. Ser.* 660, 189–201. <https://doi.org/10.3354/meps13599>.
- Parra-Torres, Y., Ramírez, F., Afán, I., Aguzzi, J., Bouten, W., Forero, M.G., Navarro, J., 2020. Behavioral rhythms of an opportunistic predator living in anthropogenic landscapes. *Mov. Ecol.* 8, 1–8. <https://doi.org/10.1186/s40462-020-00205-x>.
- Partecke, J., Schwabl, F., Winner, E., 2006. Stress and the city: urbanization and its effects on the stress physiology in European blackbirds. *Ecology* 87, 1945–1952. [https://doi.org/10.1890/0012-9658\(2006\)87\[1945:SATCUA\]2.0.CO;2](https://doi.org/10.1890/0012-9658(2006)87[1945:SATCUA]2.0.CO;2).
- Patenaude-Monette, M., Bélisle, M., Giroux, J.-F., 2014. Balancing energy budget in a central-place forager: which habitat to select in a heterogeneous environment? *PLoS One* 9, 1–12. <https://doi.org/10.1371/journal.pone.0102162>.
- Peleteiro, M.C., 2016. *Técnica de necropsia de aves*. In: Peleteiro, M.C. (Ed.), *Manual de Necropsia Veterinária*. Lidel - edições técnicas, Lda, pp. 97–104 ISBN: 978-989-752-196-6.
- Pierotti, R., Annett, C.A., 1991. Diet choice in the herring gull: constraints imposed by reproductive and ecological factors. *Ecology* 72, 319–328. <https://doi.org/10.2307/1938925>.
- PORDATA, 2011. Portuguese population censuses (in Portuguese). <https://www.pordata.pt/Subtema/Portugal/Censos+da+Populacao-27> (accessed 1 June 2021).
- Provencher, J.F., Bond, A.L., Avery-Gomm, S.M., Borrelle, S.B., Bravo Rebolledo, E.L., Hammer, S., Kühn, S., Lavers, J.L., Mallory, M.L., Trevaill, A., van Franeker, J.A., 2017. Quantifying ingested debris in marine megafauna: a review and recommendations for standardization. *Anal. Methods* 9, 1454–1469. <https://doi.org/10.1039/C6AY02419J>.
- Provencher, J.F., Avery-Gomm, S., Liboiron, M., Braune, B.M., Macaulay, J.B., Mallory, M.L., Letcher, R.J., 2018. Are ingested plastics a vector of PCB contamination in northern fulmars from coastal Newfoundland and Labrador? *Environ. Res.* 167, 184–190. <https://doi.org/10.1016/j.envres.2018.07.025>.
- Provencher, J.F., Borrelle, S.B., Bond, A.L., Lavers, J.L., van Franeker, J.A., Kühn, S., Hammer, S., Avery-Gomm, S.M., Mallory, M.L., 2019. Recommended best practices for plastic and litter ingestion studies in marine birds: collection, processing, and reporting. *Facets* 4, 111–130. <https://doi.org/10.1139/facets-2018-0043>.
- Puskic, P.S., Lavers, J.L., Adams, L.R., Grünenwald, M., Hutton, I., Bond, A.L., 2019. Uncovering the sub-lethal impacts of plastic ingestion by shearwaters using fatty acid analysis. *Conserv. Physiol.* 7. <https://doi.org/10.1093/conphys/coz017>.
- R Core Team, 2019. R: a language and environment for statistical computing. Available at: R Foundation for Statistical Computing, Vienna, Austria. <http://www.r-project.org/>.
- Ramos, R., Ramirez, F., Sanpera, C., Jover, L., Ruiz, X., 2009. Diet of yellow-legged Gull (*Larus michahellis*) chicks along the Spanish Western Mediterranean coast: the relevance of refuse dumps. *J. Ornithol.* 150, 265–272. <https://doi.org/10.1007/s10336-008-0346-2>.
- Real, E., Oro, D., Martínez-Abraín, A., Igual, J.M., Bertolero, A., Bosch, M., Tavecchia, G., 2017. Predictable anthropogenic food subsidies, density-dependence and socio-economic factors influence breeding investment in a generalist seabird. *J. Avian Biol.* 48, 1462–1470. <https://doi.org/10.1111/jav.01454>.
- Rochman, C.M., Browne, M.A., Underwood, A.J., van Franeker, J.A., Thompson, R.C., Amaral-Zettler, L.A., 2016. The ecological impacts of marine debris: unravelling the demonstrated evidence from what is perceived. *Ecology* 97, 302–312. <https://doi.org/10.1890/14-2070.1>.
- Rohart, F., Gautier, B., Singh, A., Lê Cao, K.A., 2017. mixOmics: an R package for 'omics feature selection and multiple data integration. *PLoS Comput. Biol.* 13, 1–19. <https://doi.org/10.1371/journal.pcbi.1005752>.
- Roman, L., Lowenstine, L., Parsley, L.M., Wilcox, C., Hardesty, B.D., Gilardi, K., Hindell, M., 2019. Is plastic ingestion in birds as toxic as we think? Insights from a plastic feeding experiment. *Sci. Total Environ.* 665, 660–667. <https://doi.org/10.1016/j.scitotenv.2019.02.184>.
- Roman, L., Gilardi, K., Lowenstine, L., Hardesty, B.D., Wilcox, C., 2021. The need for attention to confirmation bias and confounding in the field of plastic pollution and wildlife impacts: comment on “Clinical pathology of plastic ingestion in marine birds and relationships with blood chemistry”. *Environ. Sci. Technol.* 55, 801–804. <https://doi.org/10.1021/acs.est.0c02874>.
- Romieu, I., Castro-Giner, F., Kunzli, N., Sunyer, J., 2008. Air pollution, oxidative stress and dietary supplementation: a review. *Eur. Respir. J.* 31, 179–196. <https://doi.org/10.1183/09031936.00128106>.
- Rosenblatt, A.E., Schmitz, O.J., 2016. Climate change, nutrition, and bottom-up and top-down food web processes. *Trends Ecol. Evol.* 31, 965–975. <https://doi.org/10.1016/j.tree.2016.09.009>.
- Rousseeuw, P.J., Leroy, A.M., 2005. *Robust Regression and Outlier Detection*. John Wiley & Sons, New York.
- Ryan, P.G., 1987. The incidence and characteristics of plastic particles ingested by seabirds. *Mar. Environ. Res.* 23, 175–206. [https://doi.org/10.1016/0141-1136\(87\)90028-6](https://doi.org/10.1016/0141-1136(87)90028-6).
- Salmón, P., Nilsson, J.F., Nord, A., Bensch, S., Isaksson, C., 2016. Urban environment shortens telomere length in nestling great tits, *Parus major*. *Biol. Lett.*, 12 <https://doi.org/10.1098/rsbl.2016.0155>.
- Seif, S., Provencher, J.F., Avery-Gomm, S.M., Daoust, P.Y., Mallory, M.L., Smith, P.A., 2018. Plastic and non-plastic debris ingestion in three gull species feeding in an urban landfill environment. *Arch. Environ. Contam. Toxicol.* 74, 349–360. <https://doi.org/10.1007/s00244-017-0492-8>.
- Shochat, E., Lerman, S., Fernández-Juricic, E., 2010. Birds in urban ecosystems: population dynamics, community structure, biodiversity, and conservation. *Urban Ecosyst. Ecol. Agron. Monogr.* 55, 75–86. <https://doi.org/10.2134/agronmonogr55.c4>.
- Sorais, M., Mazerolle, M.J., Giroux, J.F., Verreault, J., 2020. Landfills represent significant atmospheric sources of exposure to halogenated flame retardants for urban-adapted gulls. *Environ. Int.* 135. <https://doi.org/10.1016/j.envint.2019.105387>.

- Spelt, A., Williamson, C., Shamoun-Baranes, J., Shepard, E., Rock, P., Windsor, S., 2019. Habitat use of urban-nesting lesser black-backed gulls during the breeding season. *Sci. Rep.* 9, 1–11. <https://doi.org/10.1038/s41598-019-46890-6>.
- Taipale, S.J., Kainz, M.J., Brett, M.T., 2014. A low ω -3: ω -6 ratio in daphnia indicates terrestrial resource utilization and poor nutritional condition. *J. Plankton Res.* 37, 596–610. <https://doi.org/10.1093/plankt/fbv015>.
- Tanaka, K., Takada, H., Yamashita, R., Mizukawa, K., Fukuwaka, M.Aki, Watanuki, Y., 2013. Accumulation of plastic-derived chemicals in tissues of seabirds ingesting marine plastics. *Mar. Pollut. Bull.* 69, 219–222. <https://doi.org/10.1016/j.marpolbul.2012.12.010>.
- Thaysen, C., Sorais, M., Verreault, J., Diamond, M.L., Rochman, C.M., 2020. Bidirectional transfer of halogenated flame retardants between the gastrointestinal tract and ingested plastics in urban-adapted ring-billed gulls. *Sci. Total Environ.* 730, 138887. <https://doi.org/10.1016/j.scitotenv.2020.138887>.
- Twining, C.W., Lawrence, P., Winkler, D.W., Flecker, A.S., Brenna, J.T., 2018. Conversion efficiency of α -linolenic acid to omega-3 highly unsaturated fatty acids in aerial insectivore chicks. *J. Exp. Biol.* 221, 1–8. <https://doi.org/10.1242/jeb.165373>.
- van Franeker, J.A., 2004. *Save the North Sea Fulmar-Litter-EcoQO Manual Part 1: Collection and Dissection Procedures*. Alterra.
- Venables, W.N., Ripley, B.D., 2002. *Modern Applied Statistics With S. Fourth edition*. Springer, New York.
- Vidal, E., Médail, F., Tatoni, T., 1998. Is the yellow-legged gull a superabundant bird species in the Mediterranean? Impact on fauna and flora, conservation measures and research priorities. *Biodivers. Conserv.* 7, 1013–1026. <https://doi.org/10.1023/A:1008805030578>.
- Wang, S.W., Iverson, S.J., Springer, A.M., Hatch, S.A., 2009. Spatial and temporal diet segregation in northern fulmars *Fulmarus glacialis* breeding in Alaska: insights from fatty acid signatures. *Mar. Ecol. Prog. Ser.* 377, 299–307. <https://doi.org/10.3354/meps07863>.
- Watson, H., Videvall, E., Andersson, M.N., Isaksson, C., 2017. Transcriptome analysis of a wild bird reveals physiological responses to the urban environment. *Sci. Rep.* 7, 1–10. <https://doi.org/10.1038/srep44180>.
- Williams, C.T., Buck, L.L., 2010. Using fatty acids as dietary tracers in seabird trophic ecology: theory, application and limitations. *J. Ornithol.* 151, 531–543. <https://doi.org/10.1007/s10336-010-0513-0>.
- Winton, R.S., River, M., 2017. The biogeochemical implications of massive gull flocks at landfills. *Water Res.* 122, 440–446. <https://doi.org/10.1016/j.watres.2017.05.076>.
- Yamashita, R., Takada, H., Fukuwaka, M.Aki, Watanuki, Y., 2011. Physical and chemical effects of ingested plastic debris on short-tailed shearwaters, *Puffinus tenuirostris*, in the North Pacific Ocean. *Mar. Pollut. Bull.* 62, 2845–2849. <https://doi.org/10.1016/j.marpolbul.2011.10.008>.
- Yorio, P., Marino, C., Kasinsky, T., Ibarra, C., Suárez, N., 2020. Patterns of plastic ingestion in Kelp Gull (*Larus dominicanus*) populations breeding in northern Patagonia, Argentina. *Mar. Pollut. Bull.* 156, 111240. <https://doi.org/10.1016/j.marpolbul.2020.111240>.
- Zeileis, A., Kleiber, C., Jackman, S., 2008. Regression models for count data in R. *J. Stat. Softw.* 27, 8. <http://www.jstatsoft.org/v27/i08/>.
- Zorrozuza, N., Egunez, A., Aldalur, A., Galarza, A., Diaz, B., Hidalgo, J., Jover, L., Sanpera, C., Castège, I., Arizaga, J., 2020. Evaluating the effect of distance to different food subsidies on the trophic ecology of an opportunistic seabird species. *J. Zool.* 311, 45–55. <https://doi.org/10.1111/jzo.12759>.