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Among-colony variation in plastic ingestion by Yellow-legged gulls (*Larus michahellis*) across the western Mediterranean basin

Florence Nono Almeida[a,*], Carole Leray[b], Charly Souc[a], Sara Scotto[b], Slaheddine Selmi[c], Abdessalem Hammouda[c], Raül Ramos[d,e], Alexandra ter Halle[f], Karen D. McCoy[a,1], Marion Vittecoq[b,1]

[a] *MIVEGEC, University of Montpellier CNRS IRD, Centre IRD, Montpellier, France*
[b] *Tour du Valat, Research Institute for the Conservation of Mediterranean Wetlands, Arles, France*
[c] *Ecology & Environment Laboratory (LR24ES17), Faculty of Sciences, Gabès University, Gabès, Tunisia*
[d] *Departament de Biologia Evolutiva, Ecologia i Ciències Ambientals, Facultat de Biologia, Universitat de Barcelona, Av. Diagonal 643, 08028 Barcelona, Spain*
[e] *Institut de Recerca de la Biodiversitat (IRBio), Universitat de Barcelona, Av. Diagonal 643, 08028 Barcelona, Spain*
[f] *Softmat, UMR 5623 CNRS - University of Toulouse III Paul Sabatier, Toulouse, France*

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ABSTRACT

The Mediterranean region is both a hotspot for biodiversity and for the accumulation of plastic pollution. Many species are exposed to this pollution while feeding, including a wide diversity of seabirds. Our objective was to investigate spatial variation in the quantity and types of plastic ingested by Yellow-legged gulls using information obtained from regurgitated pellets collected in 11 colonies. Anthropogenic debris, and particularly plastic, was found in pellets from all colonies, but the amount varied considerably. This among-colony difference was stable over the two years of study. The presence of marine prey and the proportion of agricultural area around the colonies significantly influenced the number of ingested plastics. As landfills close and garbage management improves, the availability of anthropogenic waste should decline. Following the response of gulls to these changes will be particularly useful for monitoring plastic pollution and for understanding the response of opportunistic wildlife to environmental modifications.

1. Introduction

Currently, 76 % of the worldwide discarded plastics end up in landfills, dumps or in the natural environment (Geyer, 2020). Plastics also accumulate in the environment via other anthropogenic sources such as wastewater runoff, fertilizers or plastic used in agriculture, or tire abrasion (Chae and An, 2018). Plastic debris come in all sizes, from large pieces (macroplastics) to invisible particles (nanoplastics), and include a wide range of different polymers. Once in the environment, plastic debris can move among ecosystems. For example, mismanaged waste is responsible for transferring between 1.2 and 12.7 million tons of plastic into the oceans every year, vectored through river discharge (Lebreton et al., 2017; Weiss et al., 2021). Once in the ocean, currents can create areas of plastic accumulation at sea, plastics can settle into the sediments, or can return to land, accumulating and breaking down on shorelines (Lebreton et al., 2019). As a consequence, the organisms that live in these diverse environments are exposed to a continuum of plastic debris, ranging from nanometers to several centimeters in size.

Largely enclosed and densely populated, the Mediterranean is a significant hotspot for the accumulation of plastic litter (Anastasopoulou and Fortibuoni, 2019; Compa et al., 2019; Consoli et al., 2020; Fossi et al., 2018). Indeed, the first record of accumulated surface plastic in Mediterranean waters in 1980 reported 1300 items per km^2 (Morris, 1980). In 2015, 243,853 items per km^2 were estimated (Cózar et al., 2015) suggesting a major increase in plastic pollution over time. This increase is of particular conservation concern (Fossi et al., 2018) as the Mediterranean Sea is also a biodiversity hotspot (UNEP-MAP RAC/SPA, 2010). The western Mediterranean area is home to 87 % of this biodiversity, but also has the highest level of plastic accumulation in the region (Compa et al., 2019; UNEP-MAP RAC/SPA, 2010). The Rhône is the main river flowing into the western Mediterranean, and contributes at least 0.7 tons of macroplastics per year (Castro-Jiménez et al., 2019). If

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* Corresponding author.
E-mail address: florence.nonoalmeida@gmail.com (F. Nono Almeida).
[1] These authors contributed equally.

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estimates include microplastics, this figure reaches close to 8.5 tons of plastic pollution per year.

Anastasopoulou and Fortibuoni (2019) identified 116 marine species affected by plastic ingestion or entanglement in the Mediterranean region, more than half occurring in the western Mediterranean. Among these, 10 seabird species have been recorded ingesting plastic. This ingestion may be direct because plastic items are mistaken for prey, or indirect as plastic accumulates through the trophic chain (Anastasopoulou and Fortibuoni, 2019; Kühn et al., 2020). Exposure to plastic may have a multitude of negative effects, from direct mortality via gut obstructions to reductions in body condition due to nutritional deprivation, decreased fat deposition, or toxicity associated with the chemical additives contained in plastics or with pollutants adsorbed on their surface (Fossi et al., 2018; Lavers et al., 2014; Roman et al., 2020). Recent studies have even highlighted a new disease associated with macroplastic ingestion in seabirds called "plasticosis" which results from scarring of the proventricular tissue (Charlton-Howard et al., 2023). Plastics can also vector pathogenic microbes and impact the gut microbiome with health-related consequences (Fackelmann et al., 2023). These different effects can threaten overall population viability by reducing individual survival and/or breeding success.

Susceptibility to plastic ingestion is known to vary among seabird species due to differences in feeding ecology (Clark et al., 2023; Franco et al., 2019; Ryan, 1987). For example, only 12.5 % of Great black-backed gull (*Larus marinus*) stomachs from the Gulf of Maine, USA, contained plastics (Caldwell et al., 2020); this species is a specialized marine predator, but sometimes shows opportunistic feeding behaviours. In contrast, ingestion is much higher in surface feeding seabirds such as Newell's shearwaters (*Puffinus newelli*) from Hawaii (50 % of birds with plastics in their stomach; Kain et al., 2016) or Northern fulmars (*Fulmarus glacialis*) from Greenland (86 % of birds with plastics in their stomach, van Franeker et al., 2022). The impacts of plastic may also differ among species depending on their feeding strategy; some seabird species, for instance, can expel indigestible debris found in food items in regurgitated pellets (Provencher et al., 2019). In these species, large plastics can be partially eliminated before damage is done to the stomach and intestine. For example, plastics were more frequent in the pellets of Great black-backed gulls from the Gulf of Maine, USA (23.1 %) than in their stomachs (12.5 %; Caldwell et al., 2020). Similarly, all pellets from Flesh-footed shearwaters (*Puffinus carneipes*) from New South Wales, Australia, contained plastic items with an average of 29.6 ± 16.2 items/pellet (Bond et al., 2021), whereas only 90 % had plastic in their stomachs and the average number of plastic items was 17.5 ± 44.9 per stomach (Lavers et al., 2014).

At the intraspecific level, variation in plastic ingestion and its impacts may also exist among individuals, populations and years, reflecting variation in plastic pollution in the colony area and/or different feeding behaviours. For example, spatio-temporal variation in the number of plastic items and overall plastic load has been reported in the Northern fulmar sampled across Europe, USA and Canada (Avery-Gomm et al., 2012; Baak et al., 2020; Bond et al., 2014; Donnelly-Greenan et al., 2014; Van Franeker et al., 2011). Fulmars from British Colombia, Canada were found to have a much higher average amount of plastic per bird (52.9 ± 17.2 items and 0.35 ± 0.09 g in 2009/2010; Avery-Gomm et al., 2012), than those from the Canadian Arctic (1.7 ± 1.6 items and 0.02 ± 0.03 g in 2018; Baak et al., 2020). However, for most other seabird species, there is little data on intraspecific variability in plastic ingestion.

In this study, we focus on the Yellow-legged gull (*Larus michahellis*), a common seabird of the Mediterranean region with an opportunistic feeding ecology. These large gulls use both natural and anthropogenic food items. Natural food sources include fish, crustaceans, molluscs and planktonic organisms (Mendes, 2017), whereas human-derived sources vary from fishing offal to any type of food waste found in garbage (Ramos et al., 2009a). Individuals within colonies may specialize in terms of their preferred food types, with some individuals strictly feeding on either natural or anthropogenic sources, and others adopting a more mixed diet (Duhem et al., 2005; Navarro et al., 2017). Ouarab et al., 2021 observed that the diets of Yellow-legged gulls from Algeria tended to be poorly diversified if they had high accessibility to landfills, with an overall decrease in fish consumption. The opportunistic feeding behaviour of these birds, the proximity of their breeding colonies to urban areas, and strong inter- and intraspecific competition for food (Arcos et al., 2001; Bellebaum, 2005; Bracho Estévanez and Prats Aparicio, 2019) make them highly susceptible to plastic ingestion (Lenzi et al., 2016; Lopes et al., 2021; Mendes, 2017; Ramos et al., 2009b; Stewart et al., 2020; Yorio et al., 2020). Indeed, competition can lead to poor food selection as individuals quickly gobble up what they covet, without being able to separate food from associated debris. Lopes et al. (2021) found that regurgitated pellets collected in urbanized areas of Portugal contained more plastic debris than those collected from natural areas, suggesting that the amount of plastic in pellets was directly linked to the food source used. Indeed, gulls from different colonies may use anthropogenic food sources to different degrees (Ramos et al., 2009b) and, therefore, show differences in exposure to plastics and its potential fitness consequences.

The goal of the present study was to investigate inter-colony variation in the quantity and types of anthropogenic debris ingested by breeding Yellow-legged gulls of the western Mediterranean Sea, with a particular focus on plastics. We examined this variation using information obtained from regurgitated pellets collected over two years (2021, 2022). Pellet collection is a non-invasive method that can be used to assess resource use and monitor the presence of anthropogenic debris in food materials (Bond et al., 2021). Plastics found in pellets are often large enough to confidently identify the type and thus, their potential source (AMAP, 2021). We therefore considered both the relative amount of plastic per pellet and the types of plastic found. We determined plastic polymer composition using Fourier-transformed infrared spectroscopy (FTIR) and used it to infer the source of the plastics. We tested the hypotheses that the distance to the nearest landfill, the number of accessible landfills, as well as the general environment around a colony (urban or agricultural) influence resource use by gulls and thus the amount of ingested anthropogenic debris. In the first year of study, we gathered samples from colonies at a broad spatial scale to evaluate global variation in exposure. If gulls ingest plastics in relation to their availability in the environment, we expected significant differences in exposure among colonies. In the second year of study, we focused more intense sampling on four colonies in the Gulf of Lions to investigate more local effects.

2. Material and methods

2.1. Pellet analysis

Regurgitated pellets were randomly collected from different nesting territories in eleven colonies of Tunisia, southern France and Spain (Fig. 1, Table 1) during one visit in the early breeding season of 2021 (mid-March to mid-April). In 2022, we randomly selected 30 nests in four of these colonies used for population monitoring (i.e., the islands of Medes, Planasse, Carteau, and Frioul; Table 1) that we visited at the same period to collect the pellets from a 1 m radius around each nest. Sampling in both years corresponded to the transition period from incubation to hatching, such that collected pellets should have been produced by adult birds only. Only fresh and structurally intact pellets were collected to ensure that results reflected the recent diet of individuals and to guarantee that samples were not contaminated by environmental anthropogenic debris (Provencher et al., 2019). Pellets were individually stored in labelled plastic bags until analyses.

Pellet composition was analysed following the protocol of Nono Almeida et al. (2023). After sorting, individual items from the pellets were grouped by category: natural (food items including fishes, molluscs, insects or bones from undetermined vertebrates, as well as

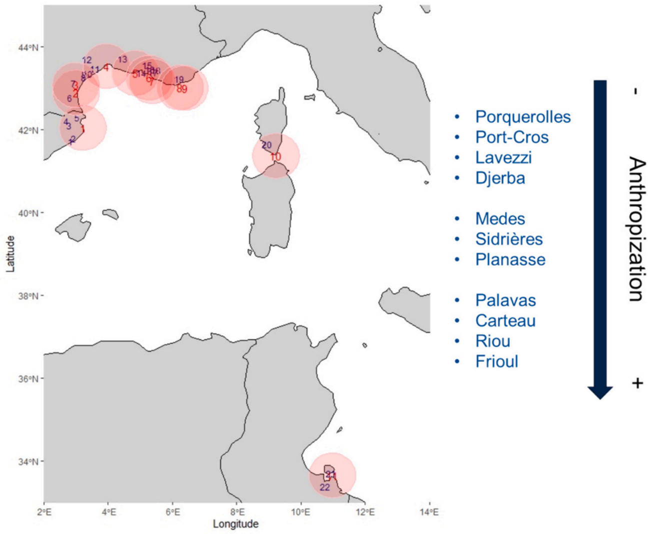

Fig. 1. Colony locations (in red) and landfills (in dark blue) within a 60 km radius from the colonies (red circle). Colony numbers refer to the list in Table 1: 1. Medes, 2. Sidrières, 3. Planasse, 4. Palavas, 5. Carteau, 6. Frioul, 7. Riou, 8. Porquerolles, 9. Port-Cros, 10. Lavezzi, 11. Djerba. Landfills: 1. GBI Serveis Lloret del Mar, 2. GBI Serveis Santa Maria de Solius, 3. Deixalleria Comarcal del Pla de l'Estany, 4. Dipòsit controlat de residus municipals de la Garrotxa, 5. Centre de Tractament de Residus Municipals de l'Alt Empordà, 6. ISDND Espira de l'Agly, 7. ISDND de Narbonne Lambert, 8. ISDND de Vendres, 9. ISDND de Béziers-St Jean de Libron, 10. ISDND de Montblanc, 11. ISDND de Villeveyrac, 12. ISDND de Soumont, 13. ISDND de Bellegarde, 14. ISDND du Vallon du Fou (Martigues), 15. ISDND la Fare-les-oliviers, 16. ISDND de l'Arbois (Aix-en-Provence), 17. ISDND Septemes-les-vallons, 18. ISDND Gardanne, 19. ISDND Pierrefeu-du-Var, 20. ISDND de Viggianello, 21. Djerba, 22. Zarzis. On the right, we indicate the gradient of anthropization of the colonies. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

indigestible natural items such as rocks, algae, shells and vegetation), anthropogenic non-plastic (cotton, glass, metal and paper) or plastic, and were weighed to the nearest 0.0001 g using a precision balance (Mettler Toledo, AG245). Based on pellet composition, a foraging habitat was assigned: marine (when fish bones, crustacean remains or any marine items were found in the pellet), terrestrial (when bones, fur, insect or any terrestrial items were found in the pellet), terrestrial and marine (when both marine and terrestrial items were found in the pellet), or unknown (when no natural item were found in the pellet and we could not determine if debris came from marine or terrestrial habitats). Details on the natural items found in the pellets are reported in Table S2 of the Supplementary Materials. Individual plastic items found in the pellets were sorted and categorized into type and colour following standardized procedures established by Provencher et al. (2017).

The chemical characterization of each plastic item was performed by attenuated total reflectance Fourier transformed infrared spectroscopy (ATR-FTIR, Thermo Nicolet 6700, Thermo Fisher Scientific) using a diamond crystal. Plastic items were gently cleaned with water and a cleanroom wipe prior to analyses and the ATR crystal was cleaned with ethanol between each particle identification (Jung et al., 2018). Infrared spectra were obtained with a resolution of 4 cm^{-1} over the wavenumber range of 400 to 4000 cm^{-1}, applying 16 scans. Each spectrum was compared with reference spectra of synthetic polymers from commercially available libraries (HR Aldrich Polymers, Polymers Miracle, HR Spectra Polymers and Plasticizers by ATR) combined into a library that we created using plastics of known composition and the OMNIC software (Thermo Fisher Scientific). A similarity threshold of 70 % was imposed to assign the chemical composition to a particle, otherwise it was considered as "non-identified" (Nono Almeida et al., 2023).

2.2. *Statistical analyses*

We collected both count and mass data during the pellet analyses. To avoid potential redundancy in our response variables, we tested for a correlation between the number and mass of both anthropogenic and plastic items per pellet using a Kendall correlation test. For the pellets

Table 1
Characteristics of sampled Yellow-legged gull colonies (location, size, number of sampled pellets, number of sampled nests, proportion of pellets with anthropogenic debris and plastic items).

Colony number (Fig. 1)	Colony name	Latitude and Longitude (in °)	Sample year	Colony size (breeding pairs)	Number of pellets	Number of sampled nests	Pellets with anthropogenic debris (%)	Pellets with plastics (%)	Reference for colony size
11	Djerba	33.653494, 10.983290	2021	~600	16	16	81.3	50	Slaheddine Selmi pers. obs. in 2021
1	Medes	42.047911, 3.221512	2021	5000	15	15	100	60	Ouled-Cheikh et al., 2021
			2022		41	14	97.6	90.2	
2	Sidrières	42.899632, 3.010122	2021	704	15	15	100	93.3	unpublished data, CEN Occitanie (2022)
3	Planasse	43.085112, 2.998192	2021	1770	16	16	100	93.8	unpublished data, CEN Occitanie (2022)
			2022		49	17	98	91.8	
4	Palavas	43.541070, 3.955867	2021	42	15	14	86.7	66.7	unpublished data, CEN Occitanie (2022)
5	Carteau	43.377640, 4.857583	2021	325	39	18	92.3	74.4	unpublished data, CEN Occitanie (2022)
			2022		54	18	90.7	77.8	
6	Frioul	43.264103, 5.288685	2021	3882	12	8	100	100	Activity report from Frioul Islands Maritime Park (2005)
			2022		73	20	95.9	58.1	
7	Riou	43.178338, 5.384021	2021	8213	9	9	100	100	Activity report from Frioul Islands Maritime Park (2005)
8	Porquerolles	43.024618, 6.240489	2021	708	15	13	100	93.3	Berger et al., 2011
9	Port-Cros	43.011053, 6.384275	2021	240	15	13	100	80	Berger et al., 2011
10	Lavezzi, Piana	41.373518, 9.228286	2021	68	10	10	80	70	Lafranchi and Faggio, in prep.

collected in 2021, there was a positive correlation between the number and mass of plastic items ($\tau = 0.50$, p-value < 0.001), although this was not the case for the number and mass of anthropogenic items per pellet ($\tau = 0.07$, p-value $= 0.22$). For the four colonies where the pellets were collected in both 2021 and 2022, we found positive correlations for both categories (respectively for anthropogenic debris and plastics: $\tau = 0.16$, p-value < 0.001; $\tau = 0.35$, p-value < 0.001). Therefore, to avoid redundancy and considering the distribution of our data, only count data were used as the response variable in subsequent analyses.

Using data from 2021, we examined whether exposure to anthropogenic debris and plastic items varied among colonies using two generalized linear mixed models (GLMM) (glmmTMB_1.1.4 R package, Brooks et al., 2017) with a negative binomial distribution due to the over-dispersion of our data. We used a forward stepwise model selection procedure and retained the most relevant variables according to the conditional Akaike Information Criterion (see the R script included in SM). The residuals of each model were evaluated to examine model fit (DHARMa 0.4.6 R package, Hartig, 2022). The full model included the following fixed effects: colony, distance to the nearest landfill, number of landfills within radius of 60 km around each colony, the presence/absence of marine items in the pellet and the percentage of agricultural coverage within 60 km of the colony, as agricultural and urban coverage were correlated (see the R script included in SM). The land cover layer was calculated using the CORINE land cover from 2018 (Copernicus, 2018). During the breeding season, gulls tend to remain near their breeding colony to forage (Mendes et al., 2018), but can travel up to 100 km per day (Ceia et al., 2014). The 60 km radius for landfills was chosen as this is the longest distance commonly covered by foraging individuals carrying GPS tags during the breeding season in the studied region (C. Souc, unpublished data). We included nest site as a random effect in models to correct for pellets collected from the same breeding pair.

We then tested whether anthropogenic and plastic exposure varied among years using the same model structure as above, but with data from the four colonies sampled in both 2021 and 2022 and including a year effect (Table 1). Given the distribution of the data, the first model considered the presence/absence of anthropogenic items using a binomial distribution with a probit link. The second model used the number of plastics as the response variable with a negative binomial distribution.

Among-colony differences in the mean number and mass of both anthropogenic debris and plastic items was assessed using Kruskal-Wallis tests, followed by a Dunn test with a Benjamini-Hochberg adjustment for multiple tests (FSA 0.9.4 package, Ogle et al., 2023). In order to explore how the type of plastic exposure varied among colonies, we also carried out a multiple correspondence analysis (MCA), considering plastic type, composition, colour, size and mass of each item (FactoMineR_2.6 and factoextra_1.0.7 R packages, Kassambara and Mundt, 2020; Le et al., 2008).

Finally, we evaluated whether the foraging habitat (marine, terrestrial or both) used by breeding gulls varied among colonies using an exact Fisher test. We first tested this using our overall data. We then tested for differences among colony pairs and corrected the p-values with a Benjamini-Hochberg adjustment for multiple tests. For these analyses, we excluded the pellets from the unknown category.

All analyses were run using the R statistical program v3.6.3 (R Core Team, 2019).

3. Results

A total of 394 pellets were collected from the 11 sampled colonies in 2021 ($n = 177$) and 2022 ($n = 217$; Table 1). At least one anthropogenic item was found in 92 % of the collected pellets and 79 % contained at least one plastic item. Details on the anthropogenic items found within each colony are reported in Table S1 of the Supplementary Materials.

Spatial variation in the number of anthropogenic items per pellet was best explained by colony and the presence of marine items in the diet (PMI), but only colony showed a significant influence when estimating the effect (see Table 2 for model selection, with more details in the R script included in SM; Table 3 gives the estimates based on the selected model). Gulls on Porquerolles, Port-Cros, Riou and Sidrières ingested a higher number of anthropogenic debris items than gulls from most of the other colonies (Fig. 2). This tendency was not observed when considering the mass of anthropogenic items in the pellets (Fig. S1A in SM).

In terms of total mass, paper was predominant, followed by plastic, metal, glass and cotton (proportionally 53.5 %, 33.6 %, 7.5 %, 4.5 % and 0.9 % of the pellet mass; see Fig. S1B in SM for a breakdown by colony). However, most pellets contained a higher number of plastic items than paper items (Dunn test paper-plastic, $z = -8.93$, p-value < 0.05); indeed, only a few pellets included large pieces of paper, whereas most pellets were loaded with many small pieces of plastic.

A total of 738 plastic items were collected (Table S1), with 2.8 ± 3.1 (SD) items per pellet on average. Colony was the main factor explaining spatial variation in the number of plastic items per pellet (Tables 2 and 3 and details in the R script in SM). As observed for the number of anthropogenic items, gulls on Porquerolles, Riou and Sidrières tended to ingest a higher number of plastic items than gulls from most of the other colonies (Fig. 3A). When considering the mass of plastic, between colony differences were less obvious; gulls on Porquerolles and Planasse ingested more plastic mass than gulls on Djerba (Fig. S2A in SM).

Ingested plastic items were mainly sheet plastic in terms of both number and mass (48.7 % and 50.4 % – see Figs. 3B and S2B in SM, confirmed with the Dunn test – see SM). FTIR characterization was very successful in identifying plastic composition; only 20 plastic items (2.6 %) could not be identified using our 70 % threshold. Polymers grouped into five main categories: polyethylene (PE), polypropylene (PP), polystyrene (PS), polyethylene terephthalate (PET) and ‘other’, composed of diverse polymers present in low frequency such as poly(ethylene:vinyl acetate:vinyl chloride) (PEVA/PVC), poly(ethylene vinyl acetate) (PEVA), polyvinyl chloride (PVC), polyurethane, nylon, and/or polyester. Plastics from pellets were mainly composed of PE (36.4 %),

Table 2

GLMM model selection results, where k refers to the number of model parameters, DEV to the model deviance, and AICc to the Akaike's Information Criterion corrected for small sample sizes. Only models with a deltaAICc (ΔAICc) of less than two compared to the minimum AICc were included in the list. The full model list, with associated results, are given in the R script in the SM. Tested variables included DNL: distance to the nearest landfill, PA: percentage of agricultural area, PMI: presence of marine items in the pellet.

Model no.	Model variables	k	DEV	AICc	ΔAICc
Data – all colonies (2021)					
Number of anthropogenic items					
11	Colony + PMI	13	707	738.1	0
1	Colony	12	709.6	738.2	0.1
Number of plastic items					
1	Colony	12	627.9	656.5	0
11	Colony + PMI	13	626.6	657.6	1.1
Data – four colonies (2021 and 2022)					
Number of anthropogenic items					
0	Null model	2	106.9	111	0
1	Colony	5	100.9	111.1	0.1
Number of plastic items					
13	PMI + PA + year	5	1228.7	1241	0
15	PMI + PA + year + DNL	6	1226.8	1241.2	0.2
10	PMI + PA	4	1231.1	1241.3	0.3
14	PMI + PA + DNL	5	1229.3	1241.6	0.6
7	PMI + colony	6	1227.3	1241.7	0.7
16	PMI + PA + year + colony	8	1225.5	1242	1
8	PMI + year	4	1232.5	1242.7	1.7
6	PMI	3	1234.7	1242.8	1.8

Table 3

Results of the GLMM examining spatial variation in the number of anthropogenic debris and plastic items per pellet from the 2021 data based respectively on model 11 and 1 (see Table 2). Details on model selection are found in SM. The reference states are Djerba for the colonies and an absence of marine items. IRR refers to the Incidence Rate Ratio and CI to the Confidence Interval.

Predictors	Anthropogenic items			Plastic items		
	IRR	CI	p-Value	IRR	CI	p-Value
Colony [Medes]	1.16	0.65–2.06	0.620	1.35	0.65–2.79	0.421
Colony [Sidrières]	3.15	1.88–5.26	**<0.001**	4.21	2.20–8.04	**<0.001**
Colony [Planasse]	2.55	1.52–4.26	**<0.001**	3.42	1.79–6.55	**<0.001**
Colony [Palavas]	1.24	0.69–2.23	0.465	1.01	0.47–2.17	0.979
Colony [Carteau]	1.12	0.63–2.00	0.704	1.00	0.47–2.12	1.000
Colony [Frioul]	1.58	0.89–2.80	0.119	2.25	1.10–4.58	**0.026**
Colony [Riou]	3.54	2.02–6.23	**<0.001**	5.15	2.55–10.39	**<0.001**
Colony [Porquerolles]	2.41	1.42–4.09	**0.001**	2.98	1.53–5.78	**0.001**
Colony [Port-Cros]	2.83	1.67–4.79	**<0.001**	3.37	1.75–6.50	**<0.001**
Colony [Lavezzi]	2.18	1.22–3.90	**0.009**	2.53	1.21–5.26	**0.013**
Presence of marine items	1.25	0.95–1.64	0.109			
Number of nests	137			137		
Number of pellets	154			154		
AICc	738.0558			656.4739		

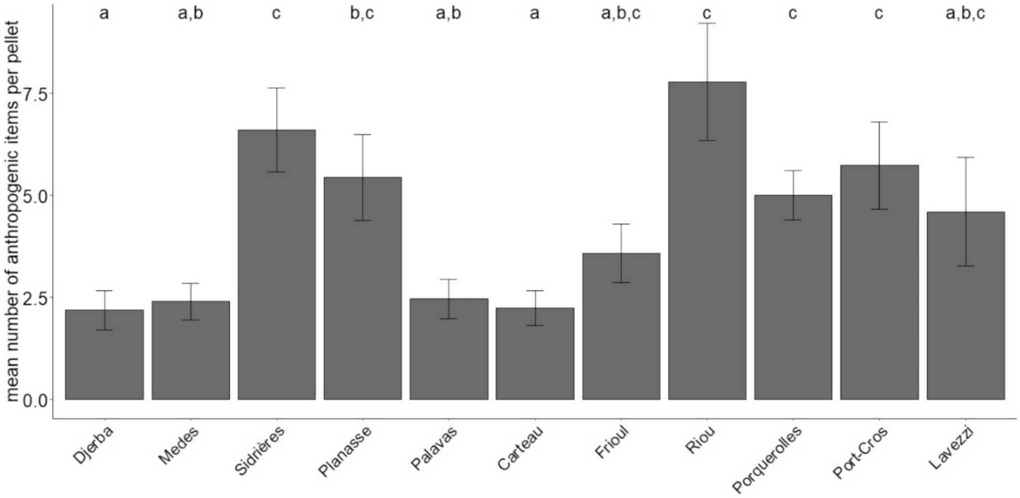

Fig. 2. Among-colony variation in mean number of anthropogenic items per pellet from the 2021 sample. The colonies with the same letter are not significantly different (Dunn test).

followed by PS (17.1 %), PP (15.4 %) and PET (8.9 %, Table 4). Only 10 % of the total variance in plastic composition among colonies was explained by the first (5.8 %) and second (4.2 %) dimensions of the MCA (Fig. 4). All colonies tended to show similar characteristics with respect to the types of ingested plastics.

When looking at the reduced four colony dataset, the top model to explain observed variation in the number of anthropogenic debris items per pellet was the null model, although a colony effect was present in a competing model (Table 2). In contrast, the selected model for variation in the number of plastic items per pellet included the presence of marine items in the pellets, the percentage of agricultural area within a 60 km radius and year (Table 2). Effect estimates were not significant for year, but the presence of marine items was associated with an increase in the number of plastic items in a pellet, and greater agricultural land coverage around the colony was related to a lower number of plastic items (Table 5).

We found a significant overall association between the colony and the foraging habitat used in 2021 (Fisher test, p-value = 0.026). However, when colonies were compared in post-hoc analyses, no significant pairwise differences were found. In general, pellet content suggested that gulls mainly fed on land, except for those in the colonies of Riou where individuals seemed to use more marine and mixed food resources, Frioul where gulls fed more on marine resources and Planasse where gulls fed more on mixed resources (Fig. 5). Details on the natural items from each habitat found in the pellets are reported in Table S2 in SM.

4. Discussion

Plastics are used for a growing number of applications in our daily lives, but their fate post-use is a major threat to the environment and biodiversity as this plastic waste permeates terrestrial and aquatic ecosystems (Auta et al., 2017; Fossi et al., 2018; Geyer, 2020; Hernandez-Milian et al., 2019; Weiss et al., 2021; Yorio et al., 2020). In our study, 92 % of collected Yellow-legged gull pellets contained anthropogenic debris, and 79 % had at least one plastic item. In terms of overall mass, plastics came second only to paper in terms of pellet content. However, while only a few individuals regurgitated large loads of paper, most gulls regurgitated at least a small amount of plastic; exposure to plastics is therefore relatively ubiquitous, as expected. However, the degree of exposure varied spatially at a large scale, with significant variation among colonies in terms of both the number of debris and plastic items per pellet.

The most frequent type of ingested plastic across colonies was polyethylene (PE). Polyethylene plastics are generally used in food packaging (Huang et al., 2020, 2021) and the remains found in gull pellets most likely come from anthropogenic waste in street garbage and

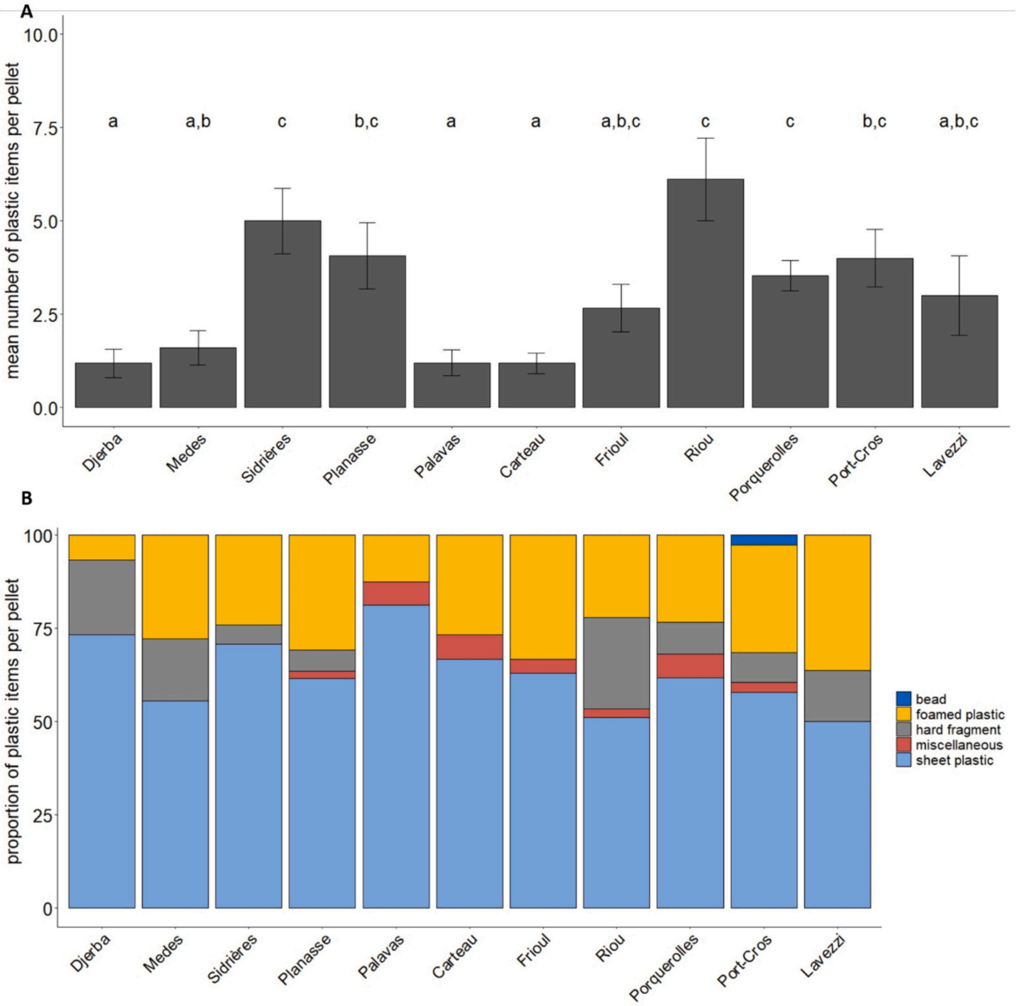

Fig. 3. Among-colony variation in pellet contents in 2021. A) The mean number of plastic items per pellet in the different sampled colonies. The colonies with the same letter are not significantly different (Dunn test). B) The plastic category breakdown based on the proportion of plastic items per pellet per colony.

landfills. These items were almost certainly ingested accidentally while gulls were eating discarded food. Indeed, as inter- and intraspecific competition can be strong in gulls (Arcos et al., 2001; Bellebaum, 2005; Bracho Estévanez and Prats Aparicio, 2019), individuals may not have time to remove food items from their packaging before ingestion.

We found no significant inter-annual variation in pellet contents between 2021 and 2022 in the four resampled colonies, suggesting at least short-term stability in exposure rates at the colony level. More data collected over longer time periods are now necessary to identify longer temporal changes in exposure rates as well as associated factors. On the long term we can expect a global increase in the amount of anthropogenic debris ingested by gulls over time given an increasing accumulation of pollution in the environment and growing human population densities (Bergmann et al., 2015). This expected increase was seen by Alonso et al. (2015), who reported that plastic exposure increased from 0.4 to 9 % between 2009 and 2011 for Yellow-legged gulls in Berlenga Island, a natural site in Portugal about 10 km from the mainland. However, a different research team working at the same site in 2018 recorded a plastic prevalence of only 1.3 % (Lopes et al., 2021). Similarly, in a previous study, we found that pellets collected in 2020 from one of the studied colonies (Carteau) showed slightly higher levels of pollution than those examined in 2021 and 2022; 93 % contained anthropogenic debris and 83.9 % had at least one plastic item (Nono Almeida et al., 2023) compared to 92.31 % and 74.4 % in 2021 and 90.7 % and 77.8 % in 2022, respectively. Such inter-annual fluctuations could be explained by several factors. Sampling error may account for some differences. Differences could also be due to among year changes in local environmental pollution levels, but we would not expect this level to change dramatically at short time scales, although plastics at sea can shift locations rapidly under different meteorological conditions (Laverre et al., 2023; Weiss, 2021). Finally, this variation could be linked to the relative availability of natural prey, such as sardine or crab, which can fluctuate strongly among years (Ceia et al., 2014), combined with the costs of foraging. The Carteau colony, for example, is located on an islet close to the small town of Port-Saint-Louis-du-Rhône (8624 inhabitants), with agricultural and port-related activities close by. There are also six landfills within the presumed foraging range of this colony (Fig. 1). The relative stability of anthropogenic resources may make them a less energetically costly food source to use than natural prey items in some years (Calado et al., 2020; Ceia et al., 2014; Moreno et al., 2010; Zorrozua et al., 2020b).

On Frioul, a colony lying approximately 3 km off-shore the city of Marseille (>860,000 inhabitants in 2021), 100 % of the pellets collected in 2021 contained plastic. A similar level of exposure to anthropogenic debris was previously reported in a Yellow-legged gull colony in Porto, Portugal (~216,000 inhabitants), with 92.5 % of the pellets containing anthropogenic debris and 90 % with plastic items (Lopes et al., 2021). The authors contrast the finding in Porto with that from pellets collected from Berlenga Island, where only 1.3 % of the pellets were found to contain anthropogenic debris, exclusively plastic items (Lopes et al.,

Table 4

Composition of plastic items found in Yellow-legged gull pellets from the different colonies sampled in 2021 and 2022, with the mean percentage of identification (±SD) and number of items per polymer type. Polyethylene (PE), Polypropylene (PP), Polyethylene terephthalate (PET), Polystyrene (PS) and Other. The unknown category refers to polymers, which could not be identified above the 70 % threshold (see methods).

Colony	Plastic composition	Percentage of identification		Number of plastic items
		Mean	±SD	
Djerba ($n = 34$ items)	PE	95.5	2.8	6
	PP	92.2	6.3	4
	PET	89.5	2.6	6
	PS	0	0	0
	Other[a]	78.4	12.3	18
	Unknown	NA	NA	0
Medes ($n = 85$ items)	PE	95.1	4.8	37
	PP	96.4	2	3
	PET	90.1	4.3	9
	PS	92.2	7.2	9
	Other[a]	83.5	7.4	24
	Unknown	NA	NA	3
Sidrières ($n = 76$ items)	PE	92.3	3.2	30
	PP	89.8	6.8	16
	PET	87.7	3	2
	PS	82.2	7.6	8
	Other[a]	80.2	6.3	19
	Unknown	NA	NA	1
Planasse ($n = 130$ items)	PE	95.3	4	46
	PP	92.5	4.6	22
	PET	88.3	3.5	11
	PS	89.9	6.4	23
	Other[a]	83.5	7	24
	Unknown	NA	NA	4
Palavas ($n = 20$ items)	PE	96.9	2.5	6
	PP	93	2.9	4
	PET	0	0	0
	PS	87.7	3.8	2
	Other[a]	89.7	3.1	6
	Unknown	NA	NA	2
Carteau ($n = 67$ items)	PE	96.4	1.5	23
	PP	94.8	4.1	9
	PET	89.7	1.5	7
	PS	90.4	10.3	9
	Other[a]	84.1	6.9	18
	Unknown	NA	NA	1
Frioul ($n = 142$ items)	PE	95.8	3.6	48
	PP	94.2	3.8	22
	PET	89	4.2	17
	PS	90	7	30
	Other[a]	84	7	22
	Unknown	NA	NA	3
Riou ($n = 56$ items)	PE	92.8	3	16
	PP	89.9	5.2	13
	PET	88.3	6.4	5
	PS	89.9	7.2	13
	Other[a]	84.8	8.2	8
	Unknown	NA	NA	1
Porquerolles ($n = 53$ items)	PE	96.2	1.9	20
	PP	93.3	2.6	6
	PET	83.4	9.9	5
	PS	90.3	7	13
	Other[a]	85.1	5.6	9
	Unknown	NA	NA	0
Port-Cros ($n = 63$ items)	PE	94.9	4.4	24
	PP	93.2	5.4	8
	PET	92.3	0.8	3
	PS	91.4	6	10
	Other[a]	80.3	8.7	15
	Unknown	NA	NA	3
Lavezzi ($n = 32$ items)	PE	96.6	1.8	13
	PP	89	6.8	4
	PET	89	NA	1
	PS	89.6	6.7	9
	Other[a]	83.4	6	3
	Unknown	NA	NA	2

[a] Poly(ethylene:vinyl acetate:vinyl chloride) (PEVA/PVC), Poly(ethylene vinyl acetate) (PEVA), Polyvinyl chloride (PVC), Polyurethane, Nylon, Polyester.

2021). A comparable natural and remote location in our study is the colony of Port-Cros, an island natural park. Like Berlenga Island, Port-Cros is heavily impacted by summer tourism (Brécard and Luigi, 2016), but is home to almost no permanent residents, although nearby islands can be occupied year-round. Interestingly, all pellets from Port-Cros had anthropogenic debris and 80 % contained plastic items. This suggests that more natural and remote habitats do not necessarily lead to lower exposure in these birds. In fact, despite the island's remoteness and conservation status, gulls from Port-Cros still have access to a landfill about 30 km from the colony.

Despite the high vagility of gulls and the presence of garbage items in the pellets of all studied colonies, the number of debris and plastic items per pellet varied spatially. When considering the full dataset of 11 colonies, neither the degree of urbanization around the colonies, nor the local availability of landfills contributed significantly to explaining this variation. However, when considering the reduced dataset of the four colonies in the Gulf of Lions sampled in both 2021 and 2022, a surprisingly positive effect of the presence of marine items (e.g., fish bones or crustacean remains) was found; that is, more plastic was associated with birds that used marine forage. This poses the question of the origin of the marine items. Discarded fish could be captured directly by gulls, but they could also be collected at open markets, in restaurant garbage bins or in landfills and, in these cases, be associated with high levels of plastics. A negative effect of the percentage of agricultural area within a 60 km radius (or a positive effect of the degree of urbanization, as these two variables were negatively correlated) on plastic exposure was also observed in this reduced dataset. Agricultural fields are used extensively during the breeding season to supply chicks with earthworms, particularly by female Yellow-legged gulls (Moreno et al., 2010; Zorrozua et al., 2020a; Zorrozua et al., 2023). Although much less polluted than urban habitats, plastics are still present in agricultural fields, as plastic mulching is frequently used to protect crop plants (Huang et al., 2020). Uptake of small plastic fragments can occur when looking for earthworms or other insects. Earthworms have also been recorded ingesting significant amounts of microplastic and nanoplastic particles (Lahive et al., 2022); these particles could not be assessed in our study, but could contribute to the plastic charge that gulls experience via accumulation through the food chain. The reported effects of marine items and land cover were only observed with the reduced dataset, and not at a broader scale. At least two alternative hypotheses could explain this difference: 1) more pellets were collected per colony in 2022 than in 2021, giving the model more power, or 2) the factors that drive plastic exposure differ across spatial scales.

As mentioned above, among-colony differences in the amount of ingested anthropogenic debris is likely related to the proximity and availability of alternative food resources and the relative costs of foraging. The availability of natural marine resources for birds has steadily declined over the last 50 years due to competition with fisheries and poor fish stock conditions in the Mediterranean sea (Grémillet et al., 2018; Vasilakopoulos et al., 2014), meaning that foraging costs may be high for some natural prey items. In contrast, landfill waste has been widely available and is even thought to be at least partially responsible for peaks in the population size of Yellow-legged gulls in the 1980s and 1990s (Alonso et al., 2015). Many birds may have switched resource use during this time, or have adopted a mixed strategy (Duhem et al., 2008). Duhem et al. (2005) highlighted that landfills were consistently used by gulls for chick provisioning in the colonies in southern France, but were not always used as main foraging habitat depending on the distance from the colony. Nono Almeida et al. (2023) found slight changes in the amount of plastic in pellets over the course of the breeding period, suggesting that if adult birds do not change foraging habitats at this time, they at least select forage items differently. Understanding the

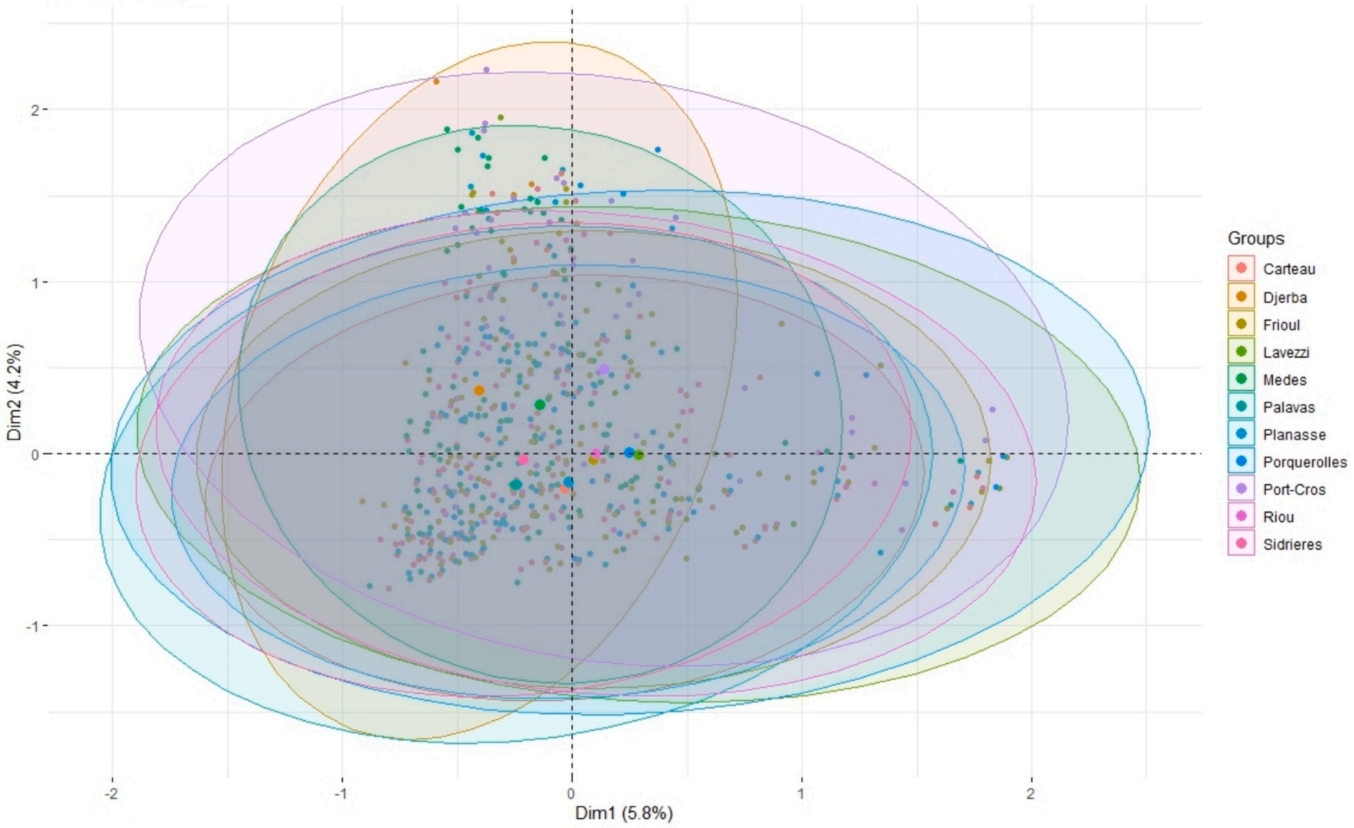

Fig. 4. Multiple correspondence analysis (MCA) comparing pellet plastic composition among colonies. Plastic composition included the morphological type, the chemical composition, the colour, the size and the mass of each item within a pellet.

Table 5
Results of the GLMM examining variation in the number of plastic items per pellet in the subset of four colonies studied over two years based on the model 13 (see Table 2). Among the top models, the presence of marine items, the percentage of agricultural area and the year were retained as explanatory variables. Details on model selection are found in SM. The reference states are the absence of marine items and the year 2021. IRR refers to the Incidence Rate Ratio and CI to the Confidence Interval. Values in bold indicate significant predictors of plastic exposure.

Predictors	Plastic items		
	IRR	CI	p-value
Presence of marine items	1.95	1.44–2.64	**<0.001**
Percentage of agricultural area	0.97	0.95–1.00	**0.047**
Year [2022]	1.25	0.95–1.66	0.117
Number of nests		125	
Number of pellets		299	
AICc		1240.967	

ability of these birds to adapt to short term changes in food availability in relation to their dietary needs should help us predict their demographic response to changes in human activities and environmental pollutants.

To date, there is limited data suggesting an obvious negative impact of exposure to anthropogenic debris on gull survival and reproduction. Indeed, Yellow-legged gulls are able to produce pellets, thus they expel most of the anthropogenic debris they ingest. However, the subtler impacts of this exposure for individual health and population dynamics remain unknown. Indeed, plastic fragments into smaller and smaller pieces over time, and may even do so when in the gull's muscular stomach. It is therefore unclear what amount of plastic, or other friable debris, may pass into the intestine. To date we know little about microplastic (<5 mm) and nanoplastic (<1 μm) accumulation in vertebrates and its potential impacts on survival and reproduction. Small plastic fragments may embed in tissues or even diffuse from the intestine to certain organs, causing inflammation and direct organ damage (Rivers-Auty et al., 2023). Moreover, the chemical additives contained in plastics to alter their physical properties and the tendency for plastics to adsorb pollutants in the environment may pose added risks for the organisms that ingest them (Liu et al., 2020; Sridharan et al., 2022). More research on these questions is needed to better estimate the impact of plastic exposure at individual and population levels.

Following recent European guidelines, the number of open-air landfills is currently declining, meaning that lower availability and higher competition for anthropogenic resources may incite Yellow-legged gulls to switch foraging habitats (Langley et al., 2021; Zorrozua et al., 2020a). Lesser black-backed gulls (*Larus fuscus*) from two colonies in the UK were found to switch to terrestrial or urban foraging habitats in the year following the closure of neighbouring landfills (Langley et al., 2021). Yellow-legged gulls from the northern Iberian Peninsula increased their consumption of terrestrial prey under the same circumstances (Zorrozua et al., 2020a). Interestingly, a monitoring study in the Balearic Islands suggested that the closure of a local landfill was responsible for a decline in body mass of adult Yellow-legged gulls, along with a reduction in egg volume and clutch size (Steigerwald et al., 2015). As anthropogenic resources decline in availability with better waste management practices, we may therefore expect to see a reduction in the exposure of gulls to anthropogenic pollution, but also an overall decline in their population demography. In fact, as the UN treaty on reducing plastic pollution in the environment moves towards a binding agreement, there is a need to focus efforts on indicators for plastic pollution. This study shows that gulls can be used as bioindicators for plastic pollution through the non-invasive collection of pellets, and are sensitive to pollution levels in the environment. Future monitoring

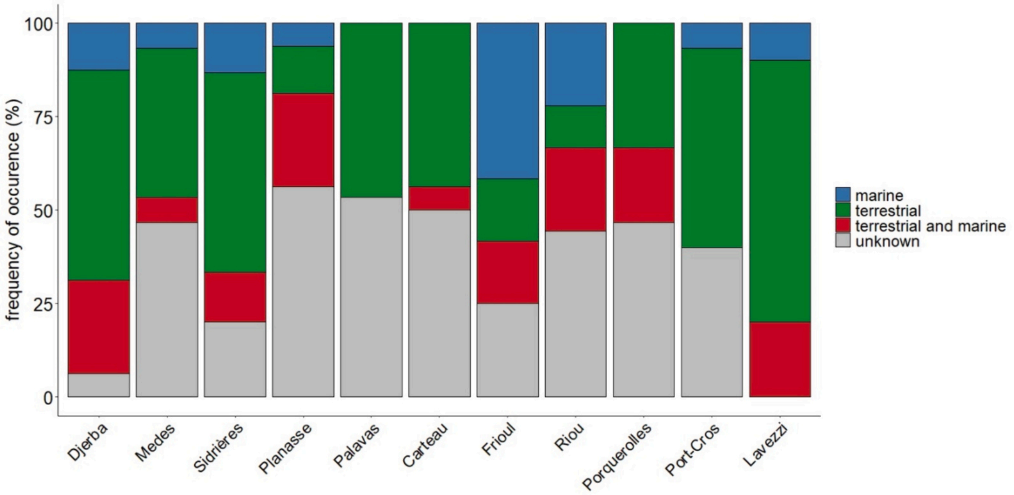

Fig. 5. Proportion of different foraging habitats used by Yellow-legged gulls in colonies sampled in 2021 based on pellet content. 'Unknown' refers to pellets that contained only anthropogenic items of unknown origin.

discussions should consider how gulls, ubiquitous across most parts of the globe, can be used to track the efficiency of policy changes to reduce plastic pollution in the environment.

In conclusion, the exposure of Yellow-legged gulls to anthropogenic pollution, and especially plastic pollution, in the western Mediterranean basin is globally high, with some inter-colony variation. GPS tracking data are now needed to further discriminate foraging habitats used by Yellow-legged gulls and to monitor switches to alternative resources with changes in the access to anthropogenic food sources. Here, we evaluated exposure to macroplastics and large microplastics, but we know little about the exposure and accumulation of smaller plastics in these birds. By understanding the overall degree and source of plastic exposure in gulls, we should be able to better estimate exposure in other species that share foraging habitats and thus anticipate the possible impact of this exposure on the overall ecosystem. However, to obtain a better understanding of the true impact of plastic population on biodiversity, work to evaluate the long-term, and potentially subtle, effects of plastic exposure on these species is now required.

CRediT authorship contribution statement

Florence Nono Almeida: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Conceptualization. **Carole Leray:** Methodology, Investigation. **Charly Souc:** Writing – review & editing, Investigation, Formal analysis. **Sara Scotto:** Methodology, Investigation. **Slaheddine Selmi:** Writing – review & editing, Investigation. **Abdessalem Hammouda:** Investigation. **Raül Ramos:** Writing – review & editing, Investigation. **Alexandra ter Halle:** Writing – review & editing, Methodology, Conceptualization. **Karen D. McCoy:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Investigation, Funding acquisition, Conceptualization. **Marion Vittecoq:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Investigation, Funding acquisition, Conceptualization.

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.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.marpolbul.2024.116508.

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