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Spatiotemporal differences in plastic biovectoring among three sympatric waterbirds

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ABSTRACT

Abiotic vectors of plastic and their impact in natural areas have been extensively studied, whereas biotic vectors have received less attention. Recent studies demonstrate that birds can act as powerful agents of plastic transport, moving large quantities of plastic from landfills to natural habitats through a process called biovectoring, causing pollution hotspots. While most studies have focused on single species, the present research broadens this approach. Here we compared the quantity, composition and spatio-temporal variation of plastic biovectoring among three co-existing waterbird species foraging on landfills near a coastal wetland: white storks, yellow-legged gulls, and lesser black-backed gulls in Cádiz Bay Important Bird Area (CBIBA; 152 km^2), Spain. We analysed 177 regurgitated pellets (42–74 per species), weighed their plastic content and used FTIR-technology to identify and classify polymer composition. We then characterized each plastic item by shape, size and colour, enabling interspecific comparisons using multiple correspondence analysis. Finally, we combined census, GPS data and the plastic obtained from the pellets to develop a daily plastic loading model for each species. We found that white storks transported the most plastic per pellet (0.14 g by median), compared to 0.034 g for yellow-legged and 0.026 g for lesser black-backed gulls. In general, gulls carried similar types of plastic items, with more film and larger sizes than storks. In total, 531 kg of plastics were estimated to be biovectored into the CBIBA from landfills in 2022, with deposition being higher in winter and lesser black-backed gulls transporting about 54 % of the total, followed by yellow-legged gulls with 30 % and white storks with 16 %. In addition, we also identified major seasonal and spatial differences among species. Our results highlight the importance of using a multi species approach to plastic biovectoring, which is essential to understand and estimate its environmental impact, and to inform management strategies.

1. Introduction

The production and use of plastics have grown exponentially since their invention in the 19th century due to their desirable physical and chemical properties (Andrady and Neal, 2009; PlasticsEurope, 2020). After use, plastics often end up in natural environments, and the ocean is the biggest environmental "sink" of plastic refuse (Cózar et al., 2014), but plastic pollution of inland waters and soils is increasingly relevant

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(Geyer et al., 2017; Windsor et al., 2018). Plastic pollution has multiple negative effects for organism and ecosystem health. Negative effects of plastic may be mechanical, such as digestive collapse, suffocation, damage to muscles, or false sense of satiety after ingestion by vertebrates (Pierce et al., 2004; Voltier et al., 2011; Wright et al., 2013; Porcino et al., 2022), perforations or fibrosis (i.e. plasticosis, Charlton-Howard et al., 2023) and entanglement (Bottari et al., 2024a), all of which may lead to mortality and reduce population size (Bletter and Wantzen, 2019; Bletter and Mitchell, 2021; Roman et al., 2022; Qian et al., 2023). Other effects come from plastic itself and its additives that are toxic and can be incorporated into cells and tissues of plants or animals, causing poisoning, oxidative stress, neurotoxicity and damaging organs (Tanaka et al., 2013; Karalija et al., 2022; Porcino et al., 2022; Rivers-Auty et al., 2023) or impairing growth (Wright et al., 2013).

The impact of plastic debris on organisms and ecosystems depends on its characteristics. For example, the nature of the polymer can determine its toxicity, persistence or origin (Bucci et al., 2020), while the shape and size of plastic fragments also determines their potential to cause entanglement, wounds, bruises, perforations or asphyxia (Wang et al., 2021; Ghaffar et al., 2022). Furthermore, the size and form of plastic items can resemble prey (e.g. fishes or worms), confusing predators and leading to their ingestion (Henry et al., 2011; Carson, 2013), whereas microplastics are often ingested unintentionally by filter feeding animals (Wright et al., 2013; Watts et al., 2014). Finally, colour may play a role in the misidentification of plastic as food (Ory et al., 2017; Noh et al., 2024), or may be related to the degree of aging and susceptibility to fragmentation, which is positively related to “lightness” (Martí et al., 2020).

Furthermore, plastics can have impacts over long distances since they can be transported by abiotic vectors such as wind, rivers, and ocean currents that move vast amounts of plastic debris (Cózar et al., 2017; van Sebille et al., 2020; González-Fernández et al., 2021). These plastics can be colonised by certain small organisms, and even used for their ecological processes (Bottari et al., 2024b; Mghili et al., 2025). In addition, animals can act as biovectors, with potential for playing a major role in the transport of plastics (Bourdages et al., 2021; Cano-Povedano et al., 2023; Martín-Vélez et al., 2024a). Pollution biovectoring occurs when birds or other animals transport contaminants from one place to another (Blais et al., 2005, 2007). This process has been described for nutrients (Hahn et al., 2007; Martín-Vélez et al., 2019), heavy metals (Martín-Vélez et al., 2021; McIntyre et al., 2022), persistent organic pollutants (Michielsen et al., 2018), antibiotic-resistant bacteria (Jarma et al., 2024) as well as plastics (Ballejo et al., 2021). Anthropogenic habitats such as open landfills are common sources of these pollutants, and are important attractors of animals (Plaza and Lambertucci, 2017; Soriano-Redondo et al., 2021; Arnold et al., 2021), where they ingest food along with environmental contaminants (Peris, 2003; Henry et al., 2011). When they return to other habitats for resting or breeding, they egest some of these plastics through their faeces and pellets (i.e. regurgitated boluses of indigestible items). Since these are regular (e.g. daily foraging trips) and directed movements (e.g. to and from the resting or breeding site), such biovectoring may lead to recurrent pollution (Grant et al., 2021; Bourdages et al., 2021; Senes et al., 2023). Plastic presence in pellets has been extensively studied and quantified for many different taxa. For instance, pellets from skuas (Hammer et al., 2015), barn owls (Petrelli et al., 2024), kingfishers (Winkler et al., 2020), vultures (Ballejo et al., 2021), cormorants (Acampora et al., 2017) or gulls (Camphuysen et al., 2008; Nono Almeida et al., 2023) have been studied looking for plastic debris.

The use of GPS devices gives vital information for the development of biovectoring models, including information about potential sources of contaminants (López-Calderón et al., 2023; Martín-Vélez et al., 2024b). GPS tracking data can also be used to calculate the proportion of individuals using these sources, so enabling the correction of census data (Cano-Povedano et al., 2023; Martín-Vélez et al., 2024a). Indeed, this information combined with regurgitation analysis has been used previously to quantify the amounts of plastics biovectored to natural areas (Cano-Povedano et al., 2023; Martín-Vélez et al., 2024a), but only for individual species. However, no previous studies have undertaken a detailed comparison of biovectoring by different bird species using the same landfill. In fact, there can be variation in the amount, polymer type and shape of plastic ingested by different waterbird species (Nicastro et al., 2018). Depending on the trophic niche, plastics found in bird pellets vary, for example fibres were common in kingfishers (Winkler et al., 2020), whereas fragments were more common in seabirds (Hammer et al., 2015; Acampora et al., 2017).

In this study, we address three coexisting waterbirds that forage at landfills while also using wetlands of Cadiz Bay Important Bird Area (Cadiz Bay IBA; Birdlife International, 2025) in southwestern Spain: The Lesser Black-Backed Gull (LBBG) *Larus fuscus*, the Yellow Legged Gull (YLG) *Larus michahellis* and the White Stork (WS) *Ciconia ciconia*. They all produce pellets to expel large items of ingested, non-digested debris, and are known biovectors (Arizaga et al., 2014; Cano-Povedano et al., 2023; Martín-Vélez et al., 2024a). These species are generalists that use inland aquatic environments, but LBBG and YLG can also exploit marine resources. LBBG and WS are full and partial migrants respectively with different phenologies, while YLG is present in Cádiz Bay the whole year. These differences in movement patterns and trophic ecology might translate into interspecific differences in plastic biovectoring.

Our main goal was, therefore, to investigate the composition, load and spatio-temporal distribution of plastic biovectored by these three species from landfills towards the wetlands in Cadiz Bay. We had four specific objectives. 1) Quantify the amount of plastic per regurgitated pellet and test which species is the most important biovector at the individual level. We predicted that WS pellets should contain more plastic than YLG and LBBG pellets, owing to their larger body size. 2) Evaluate differences in plastic type (polymer composition, shape, particle size and colour) transported by each species. We expected differences in the composition of pellets due to differences in foraging ecology among species. In particular, because of their feeding behaviour and bill size, we expect gull pellets to be more similar to each other when compared with the WS. 3) Test differences in the frequencies of visits to landfills across the three species to determine which is more likely to use any landfill while using the natural area for roosting. We expected WS to rely more on landfills than gulls, because the former cannot forage on adjacent saltwater habitats. 4) Estimate the total amount of plastic transported by each species from landfills to the wetland complex by combining pellet analyses (Objective 1), tracking and census data. In addition, we aimed to identify interspecific differences in spatio-temporal patterns of plastic transport. Because of the high abundance of gulls in the study area, we expected that gull populations would transport more plastic per annual cycle than the previously studied WS (Cano-Povedano et al., 2023).

2. Material and methods

2.1. Study area

This study was performed in a section of the Cadiz Bay IBA ES0000502, southwest Spain (Fig. 1). Our modified IBA polygon (CBIBA from hereon, 152 km^2) is mostly occupied by Cadiz Bay Natural Park and salt ponds situated nearby. They both form a complex of intertidal mudflats and solar saltworks (Camilleri, 2015) recognized as an important foraging and roosting site for migratory waterbirds (Infante et al., 2011; Bécares et al., 2019). The dominant plants in the area are *Arthrocnemum macrostachyum, Sarcocornia fruticosa, Limoniastrum monopetalum* and *Halimione portulacoides* (Junta de Andalucía. Consejería de Sostenibilidad y Medio Ambiente, 2024). In addition, we focused on two open-landfills outside the CBIBA used by gulls and storks, with maximum daily counts of 1170 WS, 9124 LBBG and 2071 YLG that move to CBIBA. These are the Verinsur (36°36′18"N 6°7′2"W) and Miramundo (36°29′2"N 6°0′53"W) landfills separated by 0.3 km and

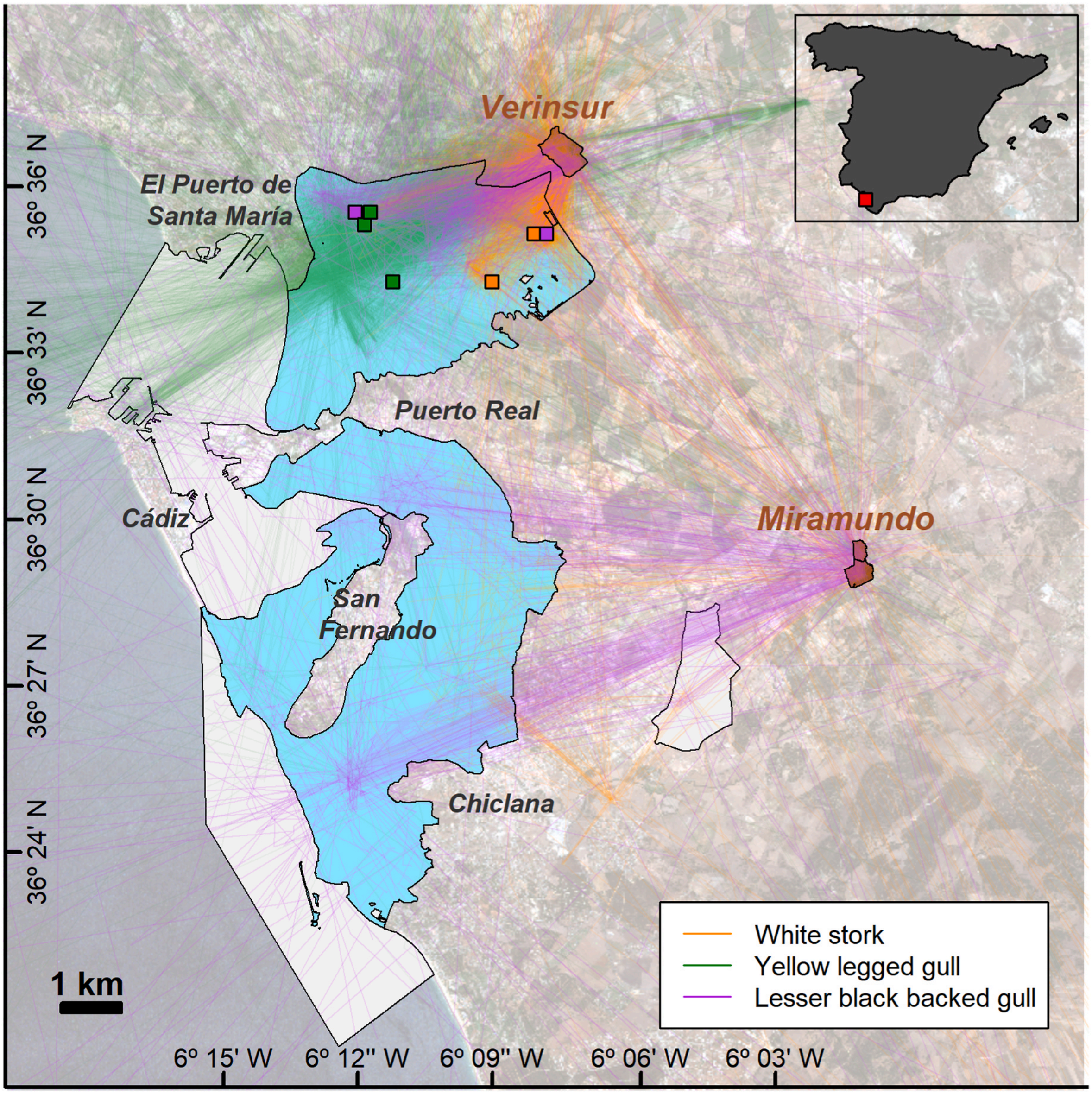

Fig. 1. Study area and individual movement tracks for each species. Bird tracks are shown with coloured lines (see inserted legend for species-specific colour codes). Brown polygons represent the two landfills (Verinsur and Miramundo), while the blue polygon represents the CBIBA and includes marshes, salt ponds, streams and dunes within and adjacent to IBA limits, based on SIPNA land use layer. White polygons are areas formally included in the IBA but eliminated from our study (marine and lagoon areas). Coloured squares show where samples were collected for each species (same colour code codes as for movement tracks).

9.3 km respectively from the CBIBA boundary (Fig. 1). The area has a Mediterranean climate influenced by strong winds, with hot summers and mild winters (Camilleri, 2015).

2.2. Sample analysis

Regurgitated fresh pellets were collected from spots where the study species roosted in monospecific groups (Martín-Vélez et al., 2022). In total, 177 pellets were collected from three species in different months from 2021 to 2023 during wintering and/or migration (detailed in Table S1, Fig. 1). The subset of WS pellets were used in a previous study (Cano-Povedano et al., 2023), but with a different method of analysis for plastics (see *FTIR analysis*). We also collected and studied faecal samples, but their contribution to the number of particles and total mass of plastic biovectored is negligible (Cano-Povedano et al., 2023; Martín-Vélez et al., 2024a), so we excluded them from further analyses.

We used aluminum foil to collect the pellet and stored them in a zip-bag, always wearing nitrile gloves. They were stored in a cool box until we arrived to the laboratory, where they were frozen (−26 °C) until processing. Pellets were then dried at 50 °C during 3–24h and placed in a glass desiccator for another 3h. We then measured dry weight (to the nearest 0.0001g on a Voyager Pro OHAUS VP214C balance) before rehydrating pellets with tap water and sieving their contents through a 0.5 mm mesh. The remaining material was placed in petri dishes and inspected through a stereomicroscope, always wearing a cotton lab coat and nitrile gloves. Anthropogenic materials were removed and classified into three categories: Highly Probable Plastic (HPP), Probable Plastic (PP) and other debris (OD), defined as anthropogenic items other than plastics (e.g. glass, ceramics). The number of particles (items) and weight was quantified for these three categories in each pellet. Items were photographed with a Nikon D3500 camera, using white paper as the background, graph paper for scale, and a colour pattern. ImageJ software (Schneider et al., 2012) was used to obtain the length and mean RGB values per item.

Each HPP or PP item was classified by size as macro- (>2.5 cm), meso- (2.5 cm–0.5 cm) and microplastic (0.5 cm–0.05 cm) following

Andrady (2017). They were also classified as (1) hard fragment (2) film, (3) line or (4) foam. Their colour was identified following Martí et al. (2020). To simplify analysis, we excluded variation in colour darkness, obtaining a final scale of 14 colours. Then, following Provencher et al. (2017) we joined them into seven groups: black, gray-silver, bluish (blue, cyan, sky, violet); greenish (turquoise, green), brownish (brown, orange), reddish (pink, red, magenta) and yellow. With this method we did not find any "off-white" item. Finally, we obtained the depth of the colours (lightness) transforming RGB values into LAB values with the *colorspace* package (Zeileis et al., 2020) in R. To explore the size distribution of plastic in further detail (beyond macro-, meso- and microplastic categories), we studied the normalized size spectrum of plastic particles following Cózar et al. (2014).

2.3. FTIR analysis

From each pellet sample, we selected representative HPP and PP items that differed in texture and colour for both categories (i.e. if several particles of plastic were apparently of similar type, we only selected one of them) and used them for FTIR analysis. At least one item per pellet was selected whenever possible.

Due to budget limitations, we made a sub-sample of HPP and PP debris. We made random staggered selections choosing one item per pellet each round, obtaining in total 126 items for WS, 126 for LBBG and 118 for YLG, corresponding to 78 items from 2021, 93 for 2022 and 199 for 2023. Finally, we successfully identified 97 items for WS, 99 for LBBG and 96 for YLG, corresponding to 58 items from 2021, 72 from 2022 and 162 from 2023. Particles were analysed by infrared spectroscopy with an FTIR instrument (Spectrum3, PerkinElmer) and a PIKE GladiATR accessory (Attenuated Total Reflectance) in the "Instituto de Investigación Marina" (INMAR) from Cadiz University. The wavenumber values ranged from 650 to 4000 cm^{-1}, and the samples were analysed with 4 scans/sample and 4 cm^{-1} nominal resolution. Background scans were performed with 32 scans/sample every ten samples. Spectra from all samples were visualised in SpectrumIR Software (PerkinElmer) and compared with spectra from the PerkinElmer Library. The mass and particles from HPP, PP and OD were corrected following Cano-Povedano et al. (2023), using the same Hit Quality Index threshold for acceptance (0.8).

2.4. Statistical analysis

All the analyses were performed in R 4.2.2 (R Core Team, 2022). To test for differences in pellet size across bird species, we performed a General Linear Model (GLM) with Gamma error distribution (link = "log") with pellet dry weight as dependent variable and bird species as the explanatory factor. To test for differences in plastic content per pellet across bird species, we used a tweedie GLM (Tweedie, 1984), with pellet dry weight (with quadratic effect) and species as predictor variables. The number of plastic particles per pellet was analysed with a negative binomial GLM including species and pellet dry weight as predictors. We compared intraspecific temporal differences in the mass of plastic in pellets with a GLM for each species, using the tweedie family, with dry weight and month of collection as explanatory factors.

To assess differences in the composition of pellets (i.e. %Plastic, %OD and %Non-anthropogenic) among species we performed compositional analysis with R package *compositions* (van den Boogaart et al., 2024). Zero values were transformed to a very small value (10^{-10}) and we transformed proportions by using the centered log-ratio method (Martín-Fernández et al., 2003). We then applied a PERMANOVA to test differences in general composition between species and a Kruskall-Wallis test to each compositional axis, with Holm-Bonferroni correction for multiple testing (Holm, 1979).

To identify interspecific differences in the characteristics (colour, size, type) of HPP/PP items we used a Multiple Correspondence Analysis using packages *factoextra* (Kassambara and Mundt, 2020) and *FactoMineR* (Lê et al., 2008). Coordinates from dimensions with more explanatory power were compared between species with Kruskal-Wallis tests. Characteristics were considered to be well represented in dimensions when $\cos^2 > 0.25$. We analysed differences in colour lightness (continuous variable from 0 to 1) among species with a GLM with family ord_beta (link = "logit"; Kubinec, 2023), with species, type of plastic and length of plastic items as predictors. To test differences among species in the frequency of occurrence of polymers egested, we used PERMANOVA (Jaccard method) using *vegan* package (Oksanen et al., 2022).

For all GLMs we used *glmmTMB* (Mollie et al., 2017), residuals were analysed with the *DHARMa* package (Hartig, 2022) and GLM post hoc tests with holm correction were applied when categorical independent variables were significant using *emmeans* package (Lenth, 2024). Dunn's post hoc tests were performed after significance in Kruskal-Wallis tests. Cragg & Uhler's PseudoR^2 values were obtained with the pR2 function from *pscl* (Jackman, 2024).

2.5. GPS data

We analysed tracking data for each bird species. For WS we used GPS data from breeding populations in Germany and Spain starting in July 2013 and ending on the 30th April 2024 (see *Data availability;* Flack et al., 2016; Fiedler, 2024a–f). For LBBG, we used movement data from breeding populations in Belgium, UK and The Netherlands starting in September 2010 (Bouten et al., 2013; Thaxter et al., 2015; Shamoun-Baranes et al., 2017; Baert et al., 2018) as well as data from Movebank starting in August 2020 (see *Data availability;* Stienen et al., 2024a,b), both datasets ending on 30th of April 2024. For YLG, we used tracking data from individuals breeding in the study area, starting in April 2022 (unpublished data) and ending on 31st May 2024.

We used the "bird-year" concept (Shamoun-Baranes et al., 2017) defined as all consecutive GPS positions of a given bird recorded during a given year. We considered that a given bird-year starts the first of June and ends the 31st May the following year, when the population size of the study species in CBIBA reaches a minimum (*see Census and Plastic Loading*, Fig. S1*)*. We only accounted for positions in the study area between 05:00–21:00h, without outliers (2D speed >80 km/h) and we resampled temporal resolution to 30 min using the *amt* package (Signer et al., 2019). For the analysis we considered only non-flying fixes (<5 km/h for WS and <10 km/h for gulls; Martín-Vélez et al., 2020; Gauld et al., 2022). Study tracks are shown in Fig. 1.

2.6. Frequency of bird visits to landfills

For each bird-year, we first counted the number of visits to any landfill as the number of days with at least one fix over a landfill and another fix over the CBIBA. To analyse interspecific differences in frequency of visits to landfills we performed a binomial GLMM ("logit" link function), with individual identity as a random intercept. Our dependent variable was the proportion of days with visits for each bird-year (excluding days without fixes over the CBIBA). We included Species and Year (from 1st June to 31st May of a given bird-year) as explanatory factors. To test differences across species in the use of each landfill, we performed a GLMM (family ordbeta) for bird-years that visited either landfill, with the proportion of landfill visits to Verinsur as response variable, species as fixed effect and individual identity as random intercept.

2.7. Censuses and plastic loading

To calculate the total amount of plastic deposited, we followed the model from Cano-Povedano et al. (2023). Census were conducted in Verinsur landfill approximately every two weeks from January 2022 to December 2022 and corrected following Cano-Povedano et al. (2023). Then, we multiplied corrected census values by the mean proportion of bird-years that move from Verinsur to CBIBA on a given day, to obtain the total number of birds travelling from Verinsur to CBIBA. WS and YLG coming from the other landfill (Miramundo) were considered 0 to simplify, because very few movements were detected. For LBBG we compared two GLMs, one for each landfill in which we used the number of bird-years in CBIBA each day as the independent variable, and bird-years travelling from Miramundo or Verinsur to CBIBA that same day as predictor. We assumed that bird numbers coming from Miramundo matched those from Verinsur since Estimate ± Standard error of the Intercept and the dependent variable overlapped. See Supplementary Material (Table S2, Fig. S2) for censuses of the three species at Verinsur landfill. We then applied the Daily Plastic Loading model described in Cano-Povedano et al. (2023), applying a span of 0.4 for LOESS estimations with 10000 simulations. In this model, each individual in a given day egests one pellet with a plastic content (including zeros) randomly selected from our pellets processed in the laboratory.

In order to identify areas that experience more plastic pollution from biovectoring by each species, we constructed heatmaps with kernelUD function from *adehabitatHR* package (Calenge, 2006). Spatial resolution of such raster surfaces was defined as 1 ha per pixel. YLG heatmaps were divided between July–October (the non-breeding season) and other November–June (breeding season). To estimate the pellets egested in each pixel, we multiplied each pixel probability by the total number of pellets egested by each species in 2022. For YLG we multiplied the number of pellets estimated to be egested by the proportion of individuals that use the landfill and breed in the northern colony of CBIBA, where birds were tagged (0.57). Finally, we calculated the percentage of pixels with overlap between species.

3. Results

3.1. Plastic content of regurgitated pellets

For all three species, ≥75 % of items in category HPP and ≥29 % of items in category PP were confirmed as plastics by FTIR (Table S3). Descriptive results from the pellet analysis are shown in Table 1A. Pellet weight differed among species ($\chi^2 = 164.31, p < 0.01$), with WS pellets being significantly heavier than those of LBBG (*Estimate* = *0.95, SE* = *0.11, t.ratio* = *8.82, p* < *0.01*), which were themselves heavier than those of YLG (*Estimate* = *0.29, SE* = *0.09, t.ratio* = *3.08, p* < *0.01*). All three species had plastics in >80 % of their pellets, with WS having the largest median plastic mass per pellet (Table 1A). However, in a GLM including bird species and pellet dry weight as predictors, the partial effect of species was not significant (Table 1B; 175 observations, $R^2 = 0.12$). In contrast, there was a strong difference between species in the number of plastic particles per pellet (Table 1B; 175 observations, $R^2 = 0.25$). Both WS (*Estimate* = *-0.61, SE* = *0.24, z.ratio* = *-2.51, p* = *0.02*) and LBBG (*Estimate* = *-0.55, SE* = *0.18, z.ratio* = *-3.17, p* < *0.01*) contained significantly more plastic particles per pellet than YLG. For WS and LBBG, there was no significant difference in the mass of plastics between pellets sampled in different months. However, there were differences between months for YLG (GLM: $\chi^2 = 10.29, p < 0.01$), with pellets from January 2023 containing more plastic than those from October 2022 (*Estimate* = *1.596, SE* = *0.540, z.ratio* = *2.959, p* = *0.01*) or February 2023 (*Estimate* = *1.755, SE* = *0.702, z.ratio* = *2.502, p* = *0.02*).

We found differences in the general composition of pellets (*F* = *3.65, p* < *0.01*) between LBBG and YLG (*F* = *4.55, padjusted* = *0.03*). After checking each pellet component, this difference was only observed for non-plastic debris (OD, $\chi^2 = 9.34, p < 0.01$, *padjusted* = *0.03*), which was heavier for LBBG than both YLG (*z-value* = *2.86, p* = *0.01*) and WS (*z-value* = *2.29, p* = *0.04*). There was no difference among species in the proportion of plastic in pellets.

All types, sizes and colours of plastics were recorded in each bird species. In all three species, brownish and gray colours dominated, microplastics-mesoplastics dominated over macroplastics, and hard fragments were the most common items (Fig. S3). In our Multiple Correspondence Analysis, the 1st and 2nd dimensions explained 22.4 % of the variance. Despite extensive overlap between species, WS was clearly different from gulls (Fig. 2 and Fig. S4). There were significant differences among species in dimension 1 ($\chi^2 = 515.05, p < 0.01$), specifically between WS-LBBG (*z-value* = *-20.50, p* < *0.01*) and between WS-YLG (*z-value* = *-16.21, p* < *0.01*), with gulls transporting more film and macroplastics than storks. Lightness differed significantly among species ($\chi^2 = 22.56, p < 0.01$), types of plastic ($\chi^2 = 117.88, p < 0.01$) and length of the item ($\chi^2 = 9.18, p < 0.01$). WS pellet items were significantly darker than those from LBBG (*Estimate* = *-0.24, SE* = *0.05, z.ratio* = *-4.71, p* < *0.01*) and YLG (*Estimate* = *-0.15, SE* = *0.06, z.ratio* = *-2.32, p* = *0.04*) (Table S4). Interspecific size spectra were quite similar, differing more at the lower and upper ends of the distributions (Fig. S5). In LBBG, the smallest microplastics were more abundant, especially compared to YLG. WS contained relatively fewer of the largest items of >10 mm length than gulls, therefore transporting fewer large mesoplastics and macroplastics.

Polyethylene and polypropylene were the prevalent polymers in all three species. The prevalence of silicone (PDMS) was higher in WS pellets, representing 14 % of identified items, while it was absent in gull pellets. The other polymers found were: polystyrene, polyester, polyethylene terephthalate, polyvinyl chloride, polyamide, polycarbonate, polycyclohexylenedimethylene terephthalate, teflon, polytrimethylene terephthalate, polyurethane, polyvinyl acetate, styrene allyl alcohol, copolymers and plasticizers. Details of numbers of items and frequency of occurrence per species and year are given in Tables S5 and S6.

The frequency of occurrence of different polymers varied between

Table 1

A) Results of pellet analyses showing the frequency of occurrence of pellets with plastic corrected by FTIR, median values of dry weight (g), median values of plastic mass per pellet (g), median number of plastic particles per pellet and median values of other debris mass (g) per pellet, with their range. B) Results of GLMs studying differences between species in: Plastic mass per pellet, with tweedie family error; Number of plastic particles corrected by FTIR per pellet, with negative binomial family error.

A)	Frequency of occurrence of plastic (Total)	Dry Weight median	Dry weight range	Plastic mass median	Plastic mass range	Plastic particles mean	Plastic particles range	OD mass median	OD mass range
WS	97.62 % (42)	9.53	3.52–33.98	0.140	0–4.16	8	0–36	0.165	0–9.80
LBBG	95.08 % (61)	3.92	0.81–9.61	0.026	0–3.47	4	0–37	0.183	0–4.97
YLG	79.73 % (74)	2.97	0.56–7.74	0.034	0–5.32	2	0–28	0.051	0–5.35

B)	GLM Plastic mass					GLM Number of particles				
	Est	SE	Z	χ^2	P	Est	SE	Z	χ^2	p
Intercept	**−3.214**	**0.603**	**−5.334**	-	**<0.001**	**1.540**	**0.257**	**5.988**	-	**<0.001**
Species	-	-	-	4.596	0.100	-	-	-	**11.243**	**<0.001**
LBBG	0.653	0.427	1.525	–	0.127	−0.053	0.231	−0.231	–	0.817
YLG	**1.003**	**0.470**	**2.134**	–	**0.032**	**−0.608**	**0.242**	**−2.511**	-	**0.012**
DW	**0.342**	**0.082**	**4.162**	**17.319**	**<0.001**	**0.071**	**0.019**	**3.679**	**12.708**	**<0.001**
DW^2	**−0.008**	**0.003**	**−3.032**	**9.191**	**0.002**	-	-	-	-	-

Intercept includes White Stork for Species variable, DW = dry weight. SE = standard error.

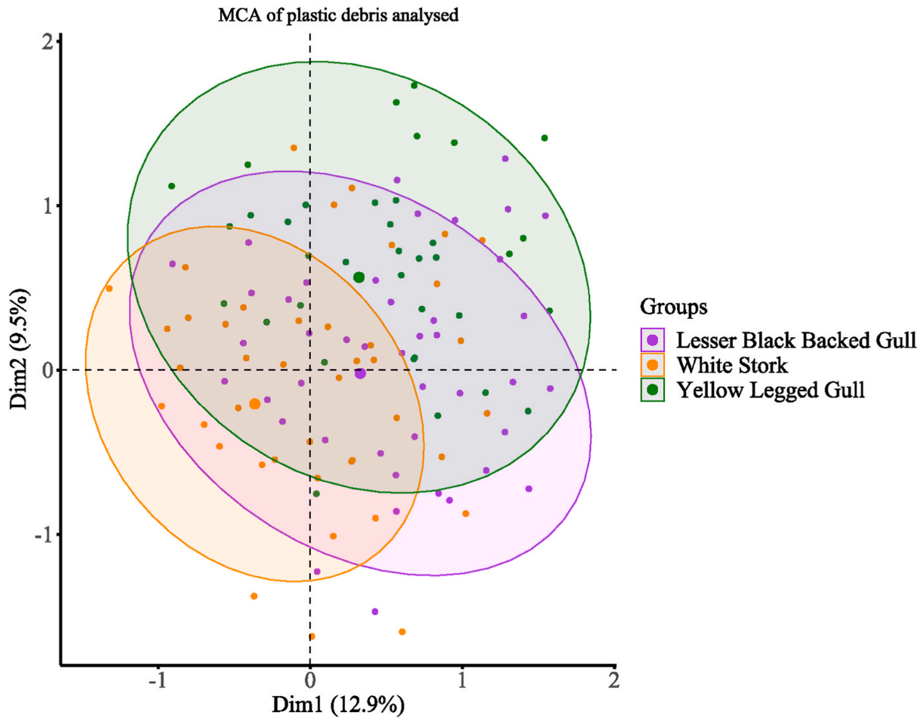

Fig. 2. Multiple Correspondence Analysis comparing items plastic composition in pellets across three bird species. Points represent the position of plastic items in the first two dimensions.

species (*F = 3.12, p < 0.01*). We found differences between WS and LBBG (*F = 3.27,1 p = 0.02*), WS and YLG (*F = 2.03, p = 0.04*) and between LBBG and YLG (*F = 4.05, p = 0.01*). These differences were explained by the high frequencies of polypropylene and polyvinyl chloride in LBBG pellets, of polystyrene and PDMS in WS, and of polyethylene in YLG (Table S5).

3.2. Landfill attendance

For a given bird-year, LBBG spent the fewest days in CBIBA, and YLG the most (Table 2A). Similarly, YLG made the most visits per bird-year to landfills, and LBBG the least, and only LBBG made more visits to Miramundo than to Verinsur (Table 2A). We found important variability among conspecific individuals in the frequency of landfill visits and landfill selection (as shown by differences in R^2marginal *vs* R^2conditional). There were differences between species in the proportion of days (while present in the CBIBA) per individual when landfills were visited (*191 bird-years and 137 individuals,* R^2*marginal = 0.37;* R^2*conditional = 0.99,* $\chi^2 = 36.65$*, p < 0.01*). WS visited landfills more often than YLG (*Estimate = 1.10, SE = 0.51, z.ratio = 2.16, p = 0.03)*, which in turn visited them more often than LBBG (*Estimate = 1.69, SE = 0.58, z.ratio = 2.92 p < 0.01*). Differences among species in landfill preference were significant (*139 bird-years and 95 individuals,* R^2*marginal = 0.48;* R^2*conditional = 0.97,* $\chi^2 = 14.21$*, p < 0.01*). In proportion, more visits were performed to Verinsur than Miramundo by YLG than WS (*Estimate = 1.19, SE = 0.42, z.ratio = -2.86 p < 0.01*), which in turn visited Verinsur more than LBBGs (*Estimate = 0.80, SE = 0.36, z.ratio = 2.23, p = 0.03*).

3.3. Plastic deposition modeling

Most of the bird-years detected in Verinsur moved to CBIBA within the same day, and to estimate biovectoring from this landfill to CBIBA we multiplied landfill censuses by 0.78 for WS, 0.90 for LBBG and 0.99 for YLG. Temporal patterns of visits and therefore plastic biovectoring varied between species (Fig. 3, Fig. S2). WS showed a bimodal distribution of population size in the study area, with autumn and spring migration peaks accounting for 1170 (15th August) and 561 (21st January) estimated individuals, and 0.65 kg and 0.31 kg of plastic transported from the landfill each day, respectively. On the other hand, the number of estimated LBBG was unimodally distributed during winter, reaching a maximum of 9124 individuals (25th January) with a

Table 2

Differences between bird species in study area use and resultant biovectoring. A) Summary of bird-years over the study area, and mean number of days per bird-year with GPS fixes at CBIBA, Miramundo and Verinsur landfills. B) Annual estimates for debris loading models, obtained using daily plastic deposition models for plastic mass, number of plastic particles, and mass of other debris (OD). Range for plastic mass, plastic particles and OD represents the minimum and maximum values of plastic and OD deposited year^{-1}.

	A) Study area use				B) Annual loading								
	Bird-Years	Days in CBIBA	Days in Miramundo	Days in Verinsur	Plastic mass (kg)	Plastic mass range (kg)	Plastic kg/km^2	Plastic Particles (million)	Plastic particles Range (million)	Plastic particles/km^2	OD mass (kg)	OD mass range (kg)	OD kg/km^2
WS	97	22.98	0.82	19.32	86.10	66–109	0.57	1.696	1.486–1.920	11150	94.99	60–143	0.62
LBBG	64	9	3.45	2.73	284.92	249–324	1.87	5.596	5.235–5.978	36788	602.46	547–662	3.96
YLG	28	283.57	2.04	168.89	160.33	123–204	1.05	1.372	1.181–1.586	9019	213.86	164–270	1.41
Total	–	–	–	–	531.35	438–637	3.49	8.664	7.902–9.484	56957	911.31	771–1075	5.99

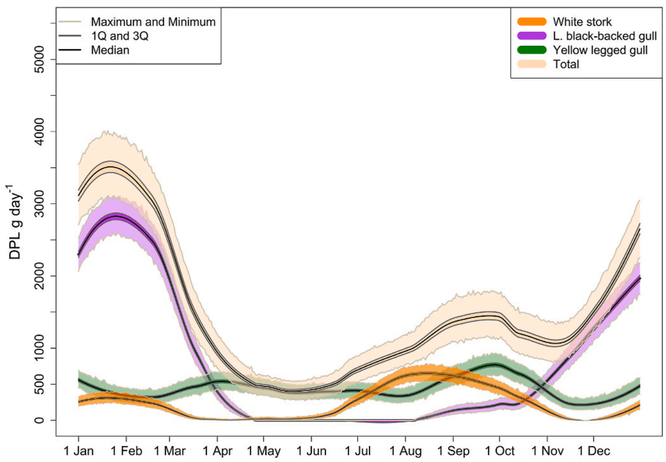

Fig. 3. Daily plastic loading deposited by species and total accumulated per day across all species during the year 2022.

maximum of 2.83 kg day^{-1} of plastic, followed by a long absence during summer. Seasonal differences were less pronounced in YLG, with maximum population size estimated at 2071 individuals (28th September), that transported 0.77 kg day^{-1} of plastic.

In total, we estimate that 531 kg of plastic were deposited in CBIBA during 2022, with 54 % contributed by LBBG, 30 % by YLG and 16 % by WS. Such plastic biovectoring accounted for an estimated 3.49 kg/km^2 over the 12 months. We also modelled the annual number of plastic particles $\geq$0.5 mm and mass of OD biovectored (details in Table 2B). The spatial distribution of plastic deposition for the three species is shown in Fig. 4. Overlap in the heatmaps was relatively low, with each species depositing debris in different areas. WS-LBBG shared 24.16 % of pixels (hectares), YLG-LBBG shared 22.21 % and WS-YLG shared only 10.39 %.

4. Discussion

Our study demonstrates the significant role that waterbirds can play as plastic biovectors. To our knowledge, this is the first study that quantifies and compares the biovectoring role of plastic concurrently and across different species within the same area. Furthermore, most research has focused on the plastic content inside their digestive tract (Holland et al., 2016; Zhao et al., 2016; Rao et al., 2021), often using dead individuals, where large debris items are more likely to be observed. While these studies demonstrate plastic ingestion, they are not useful to quantify how much plastic birds potentially transport across environments (Provencher et al., 2019).

4.1. Size matters: differences in the abundance of plastic in pellets

The mass of plastics egested in pellets does not directly depend on the species but is related to the size of the pellets. As we hypothesized, the bigger species had bigger pellets and also contained more plastic in the pellets. In consequence WS is the most important biovector in terms of quantity per cápita per day. In contrast, YLG contained fewer particles per pellet than the other two species, probably caused by the lack of debris in some of their pellets. We found differences between proportions of different materials in pellets, with a higher proportion of OD in LBBG, for unknown reasons. If this was due to variation in foraging behaviour, we would also expect differences between WS and YLG, which was not the case. One possible explanation might be differing availability of these materials in each landfill, since LBBG used Miramundo as well as Verinsur, but we lack data on the availability of materials at each landfill. The mean amount of plastic found in LBBG pellets in Fuente de Piedra (Martín-Vélez et al., 2024a) was 0.29g, very similar to our 0.31g of plastic mass per pellet, suggesting that biovectoring per cápita could be similar between different landfills.

4.2. Species differences in the composition of plastic in pellets

In general terms, plastic characteristics (type, colour, size) were more similar between gull species. In WS we found relatively more microplastics (0.5–5 mm) and fewer meso- and macroplastics. This seems counter-intuitive due to the larger body, gape and bill size of WS, but could perhaps be related to more break down of larger particles in the heavier gizzard of WS, or less capacity of gulls to egest smaller plastics in their pellets, with potential impacts for their health (Schutten et al., 2024). We also found more fragments in WS and more film particles in gulls, but it is hard to separate this from the lightness of plastic because hard fragments are usually darker, and films are lighter. These results may be driven by certain dietary preferences, e.g. gulls might identify food inside lighter-film plastics, typically used for packaging, more readily. On the other hand, WS may select dark-hard fragments that resemble insects, which are far more common in WS than in gull pellets (personal observation). Related studies with different bird species such as barn owls and kingfishers found mostly fibres in their pellets (Winkler et al., 2020; Petrelli et al., 2024), in contrast to our species that mostly transported fragments. Fragments are common in other waterbirds and seabirds like skuas (Hammer et al., 2015) or cormorants (Acampora et al., 2017) where fibres were absent or rare. This variation might be caused by the source of the plastics or how they swallow food. However, differences in the particle sizes analysed might also be the explanation, with fibres dominating in the smaller range of plastics.

Smaller plastic particles can be ingested by detritivorous filter-feeding aquatic invertebrates (Gallitelli et al., 2024; Cesarini et al., 2025) and liberate relatively more additives due to their bigger proportional surface area (Wright et al., 2013; Ghaffar et al., 2022). Furthermore, lightness plays an important role in degradation (Zhao et al., 2022), with darker plastics showing less tendency to degrade. The smaller plastic particles biovectored by WS might therefore be ingested more readily by other filtering species, whereas items from gull pellets may suffer faster degradation once released into the environment due to their lightness. Interestingly, the size-spectra we recorded was similar to that of floating plastic items from marine waters, and plastic pollution biovectored by birds is not very different from the better-known surface marine plastic pollution in terms of types, sizes and polymers (Cózar et al., 2015; Erni-Cassola et al., 2019).

The differences in prey selection mentioned above might also explain why PDMS (silicone, not a plastic in the strictest sense) was abundant only in WS pellets and was never recorded in gulls. This difference was previously observed in the stomach contents from dead birds (Nicastro et al., 2018). Although the presence of this material in storks was attributed to the similarity of rubber bands with worms (Henry et al., 2011) silicone did not appear in samples from gulls, that also feed on worms. Polypropylene and polyethylene were common in pellets for the three species, in line with previous studies (Nicastro et al., 2018; Martín-Vélez et al., 2024a) probably due to their general abundance, representing 45% of the plastic produced globally in 2022 (PlasticsEurope, 2023). These two major plastic polymers biovectored by our study species have important impacts (see review by Rodrigues et al., 2019) such as growth inhibition, reduction in body size, oxidative stress or developmental defects. Polystyrene, another common plastic among our samples can produce similar impacts as well as reduction in cerebral catalase activity (de Souza et al., 2022).

In contrast to our results, previous research found interspecific differences in plastic colours, showing proportionally more white-clear colours in YLG and more black ones in LBBG (Basto et al., 2019; Nono Almeida et al., 2023). However, this could be explained by different methodologies. Our standardised method based on RGB values (Martí et al., 2020) captures a broader range of colours, distinguishing lighter grays and brown colours from white and darker hues from black.

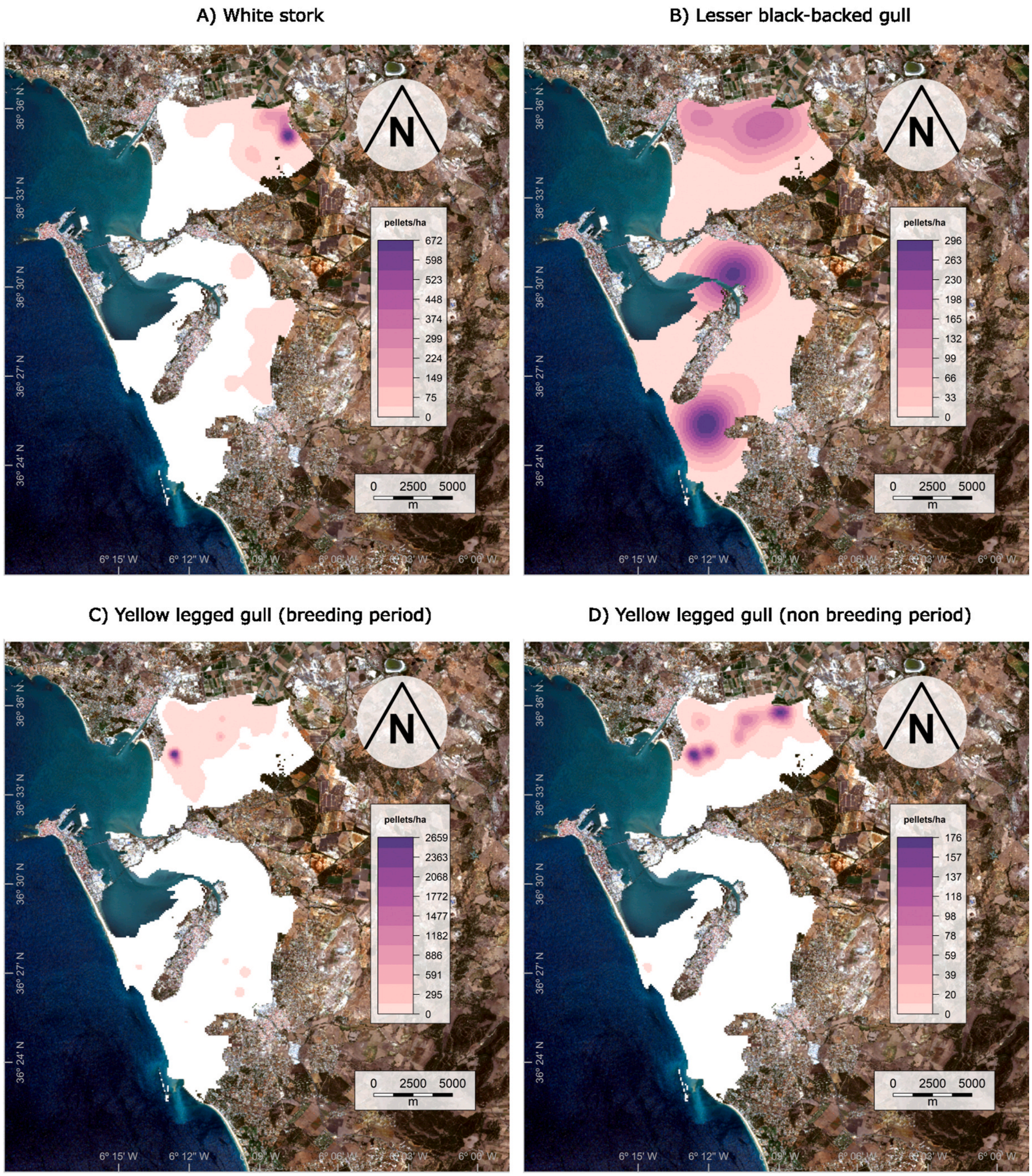

Fig. 4. Kernel Utilization Distributions pooling together all fixes for each GPS-tagged species. Probability of occurrence was converted to pellets per hectare based on total pellet load we estimated for each species in 2022, and we kept only pixels with ≥ 1 pellet estimated. A) White stork (WS), B) Lesser black-backed gull (LBBG), C) Yellow legged gull (YLG) colony tagged during the breeding season (from November to June). D) Yellow legged gull north colony during the rest of the year (from July to October). Based on a Raster Image from Sentinel-2.

4.3. Reliance on landfills

In concordance with our initial hypothesis, the most faithful visitor to landfills was the WS. This species has modified its migration pattern due to landfill presence (Flack et al., 2016) and is highly dependent on dumps for foraging (Soriano-Redondo et al., 2021, López-Calderón et al., 2023). As each WS is more likely to visit a landfill than a gull, their potential biovectoring role per cápita is also larger. In contrast, gulls can better exploit marine and urban resources, reducing the dependence on landfills. The lower proportion of pellets with plastic in YLG suggests that in our study area they forage in other habitats besides landfills. In contrast to WS and YLG, LBBG individuals that visited CBIBA used the Miramundo landfill for foraging more frequently than the Verinsur landfill, probably because, as shown by heatmaps, they also use areas in CBIBA closer to Miramundo than Verinsur, in line with an energy-saving strategy (Langley et al., 2021).

4.4. Differences in plastic load through the year

Even though WS transported more plastic per individual, overall LBBG introduced most of the plastic into the CBIBA because they were present in large numbers during winter. The LBBG is one of the most abundant wintering waterbirds in SW Spain, although their breeding populations are decreasing recently across most of Europe (Wetlands

International, 2025). Beyond the two landfills studied here, plastic biovectoring by this species is a widespread environmental issue in Andalusia (Martín-Vélez et al., 2020, 2024a) and probably wherever this species winters across southern Europe. Although a similar loading model was first developed to be used with WS, their use can be extended to many other species so long as it is possible to estimate the number of individuals moving to the natural area, and the number of pellets egested by that species. However, the intraspecific temporal differences obtained in plastic estimations deposited from our model are driven by the number of birds, and we did not consider the possibility of temporal variation in the plastic content per pellet. This is a potential limitation of our approach. It is possible there may be temporal differences in pellet composition, particularly if there are changes in the availability of plastic in the landfill, or differences in behaviour between migratory or breeding periods. While Nono Almeida et al. (2023) found differences in the plastic ingestion of YLG during breeding periods, other gull species have shown little differences in the plastic ingestion along the whole year (Jardine et al., 2021). We assumed that pellets contained a similar amount of plastic independently of the month, and only found evidence for temporal variation in the case of YLG. Ideally, sampling at more regular intervals would further assess intraspecific variation and therefore improve the plastic loading model.

Martín-Vélez et al. (2024a) estimated a mean of 400 kg of plastics deposited each winter by LBBG in Fuente de Piedra (Spain, 1476ha) and specifically around 200 kg in 2022. These values are quite similar to our estimations of plastics transported from landfills by LBBG, with a range of 249–324 kg of plastics biovectored in 2022. Previous studies have focused on the number of particles transported by birds (Bourdages et al., 2021; Senes et al., 2023) but it is not possible to compare them with our estimations since minimum size limits are not equivalent or not even mentioned. We propose the use of total plastic mass as a better proxy than particle abundance to analyse biovectoring, since mass allows a more accurate and relevant comparison among species and studies, whilst standardizing for a better comparison (i.e. controlling for sample size and surface area).

YLGs included in our GPS dataset were captured while breeding in a colony at the north of CBIBA. Although the species also breeds in south of the CBIBA with a total of 2970 breeding pairs, 1095 breed in the north (Junta de Andalucía. Consejería de Medio Ambiente, 2004). Therefore, our YLG heatmaps are only representative of birds breeding in the north (i.e. pellets estimated to be deposited in the south are not shown in Fig. 4). The heatmap might be also biased by the exact positions of the nests tagged. However, all the nests in the northern colony belong to the north-western part, close to the hotspot estimated. Due to the abundance of plastics deposited by pellets around the colonies and the use of plastic as material for nest construction in YLG (Martín-Vélez et al., 2024c) we can conclude that the areas most affected by plastic pollution will be those used for nesting by this species, and therefore more attention should be paid to them when considering likely impacts.

The spatio-temporal differences between distributions of plastics deposited into the Cádiz Bay by the three species demonstrate the importance of our multispecies approach to produce reliable and detailed estimates of plastic inputs to an ecosystem. This reinforces the importance of minimizing the biovectoring problem by improved waste and landfill management. Deterrence methods in landfills (e.g. falconry or pyrotechnics) are common methods to avoid bird presence, but reliance on a single method has limited success, and a combination of them is usually more useful (Baxter and Robinson, 2008; Castège et al., 2015; Martín-Vélez et al., 2024a), especially when several target species are involved.

4.5. Beyond plastic biovectoring, effects in ecosystems

Plastic is present not only in pellets but also in nests of many different bird species (Voltier et al., 2011; Gallitelli et al., 2023), including YLG (Martín-Vélez et al., 2024c) that breed in the area. The presence of plastics in nests might affect the survival of chicks (Heinze et al., 2025) and be a different source of plastic biovectoring to natural areas.

The roosting and breeding areas (in the case of YLG) used by gulls and storks comprise marshes, abandoned salt ponds and aquaculture areas with insects, crabs, fishes and many other migratory bird species (Perez-Hurtado and Hortas, 1993; Infante et al., 2011) that are all susceptible to plastic ingestion (Watts et al., 2014; Bajt, 2021; Flemming et al., 2022) affecting the health of the ecosystem beyond that of the biovector itself. Furthermore, biovectoring can have indirect effects caused by pollutants associated with plastics. Plasticizers, flame retardants, metals, antibiotic resistant bacteria, or other contaminants are potentially adsorbed (Teuten et al., 2009; Velzeboer et al., 2014; Liang et al., 2023) and can affect birds that ingest those plastics (Tanaka et al., 2020). Indeed, although these biovectors are able to egest most of the plastic in pellets, they might be absorbing these associated contaminants, resulting in health impacts (Sühring et al., 2022; Kerric et al., 2023; Veríssimo et al., 2024). Landfills are also hotspots of antimicrobial resistant bacteria (ARBs) and genes (ARGs, Jarma et al., 2024). The spread of ARBs and ARGs from landfills into other habitats by birds is another problem with important implications for human health (Martín-Vélez et al., 2024b; Sacristán-Soriano et al., 2024). Although most ARBs are dispersed in faeces and not pellets, our modelling gives a clear indication of the potential temporal and spatial biovectoring of antimicrobial resistance by three bird species in the CBIBA.

5. Conclusions

Combining GPS tracking, censuses, and pellet analysis, we showed that plastic transported by three species differs in quantity, composition and spatio-temporal distribution. We found that although storks transported more plastics per capita, the more abundant LBBG transported more mass of this contaminant in total. Pellets from the two gulls contained a more similar plastic composition compared to those from storks. We found that no single species acts as a “sentinel” predicting the patterns of other species, and research into plastic biovectoring into natural ecosystems requires the study of all the main species involved. Furthermore, improved harmonization of methods is necessary to facilitate future comparison of plastic loading between species, and between study areas, in biovectoring studies. Plastic biovectoring into natural habitats by birds is a problematic consequence of human deposition of plastic and food in open landfills. The closure of open landfills has been regulated in the European Directive 1999/31/UE, which might help to reduce the impact of these biovectors. Nevertheless, it is also important to consider alternative strategies. For instance, a better separation of organic residues and plastics could reduce plastic intake in birds, and could also be applied away from landfills, in other potential sources of avian plastic ingestion (e.g. urban areas or ports).

CRediT authorship contribution statement

Julián Cano-Povedano: Writing – review & editing, Writing – original draft, Visualization, Software, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Cosme López-Calderón:** Writing – review & editing, Validation, Supervision, Software, Methodology, Formal analysis, Data curation, Conceptualization. **Francisco Hortas:** Writing – review & editing, Investigation, Funding acquisition. **Victor Martín-Vélez:** Writing – review & editing. **Marta I. Sánchez:** Writing – review & editing, Supervision, Conceptualization. **Belén Cañuelo-Jurado:** Writing – review & editing, Investigation. **Andrés Cózar:** Writing – review & editing, Methodology, Conceptualization. **Judy Shamoun-Baranes:** Writing – review & editing, Resources, Data curation. **Wendt Müller:** Writing – original draft, Resources. **Chris B. Thaxter:** Writing – review & editing, Resources. **Luc Lens:** Writing – review & editing, Resources. **Eric Stienen:** Writing – review & editing, Resources. **Manuela G. Forero:** Writing – review & editing, Resources, Funding acquisition. **Isabel Afán:** Writing – review & editing,

Resources. **Julio Blas:** Writing – review & editing, Resources. **Wolfgang Fiedler:** Writing – review & editing, Resources, Data curation. **Andy J. Green:** Writing – review & editing, Validation, Supervision, Project administration, Methodology, Funding acquisition, Formal analysis, Conceptualization.

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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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envres.2025.122477.

Data availability

Data will be made available on request.

Datasets analysed during the current study are available in Movebank Data Repository (https://www.movebank.org), White stork downloaded on 19th June 2024: Movebank studies named LifeTrack White Stork Bavaria (ID: 24442409), LifeTrack White Stork Rheinland-Pfalz (ID: 76367850), LifeTrack White Stork Oberschwaben (ID: 212096177), LifeTrack White Stork Sarralbe (ID: 1562253659), LifeTrack White Stork Vorarlbeg (ID: 173641633), LifeTrack White Stork: SW Germany: (ID: 21231406), LifeTrack White Stork Donana (ID: 9648615)

For Lesser black-backed gull downloaded from Movebank on 20th January 2025: DELTATRACK - Herring gulls (*Larus argentatus*, Laridae) and lesser black-backed gulls (Larus fuscus, Laridae) breeding at Neeltje Jans (Netherlands) (ID: 1258895879), LBBG_Juvenile: LBBG_JUVENILE - Juvenile lesser black-backed gulls (Larus fuscus, Laridae) and herring gulls (*Larus argentatus*, Laridae) hatched in Zeebrugge (Belgium) (ID: 1259686571).

UvA-BiTS tracking data (https://www.uva-bits.nl) downloaded on 19th June 2024: LBBG_WALNEY, LBBG_SKOKHOLM, LBBG_ORFORDNESS, LBBG_SCHIERMONNIKOOG; LBBG_TEXEL, LBBG_ZEEBRUGGE, LBBG_IJMUIDEN

YLG data downloaded on 30th June 2024.

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