

## Biovectoring of plastic by white storks from a landfill to a complex of salt ponds and marshes

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### ABSTRACT

Research into plastic pollution has extensively focused on abiotic vectors, overlooking transport by animals. Opportunistic birds, such as white storks (*Ciconia ciconia*) often forage on landfills, where plastic abounds. We assess plastic loading by ingestion and regurgitation of landfill plastic in Cadiz Bay, a major stopover area for migratory white storks in south-west Spain. On average, we counted 599 storks per day moving between a landfill and a complex of salt ponds and marshes, where they regurgitated pellets that each contained a mean of 0.47 g of plastic debris, dominated by polyethylene. Modelling reliant on GPS tracking estimated that 99 kg and >2 million particles of plastic were biovectored into the wetland during 2022, with seasonal peaks that followed migration patterns. GPS data enabled the correction of field censuses and the identification of plastic deposition hotspots. This study highlights the important role that biovectoring plays in plastic transport into coastal wetlands.

### 1. Introduction

Pollution is one of the main drivers of Global Change and has a particularly important impact in aquatic environments (Cózar et al., 2014; Bletter and Wantzen, 2019; Morales-Caselles et al., 2021; Jaur-eguiberry et al., 2022). The presence of contaminants such as metals, pharmaceuticals or plastics in natural ecosystems is increasing due to human activities (Hampel et al., 2015; Zalasiewicz et al., 2016). These anthropogenic inputs may have lethal and sublethal effects on organisms, including oxidative stress, impaired growth or reduced reproductive success (Zhao et al., 2016; Ghaffar et al., 2022; Roman et al., 2022).

Global plastic production reached almost 391 million tonnes in 2021, of which 57.2 million tonnes were produced in Europe, the third biggest producer after Asia and North America (PlasticsEurope, 2022). The use of plastics has spread rapidly because of their low-cost and resistance to biological and chemical degradation (Zalasiewicz et al., 2016). However, this makes them highly persistent within natural ecosystems, so over time plastics break down into smaller particles, which can be assimilated and subsequently incorporated into the food chain

(Zalasiewicz et al., 2016; Sendra et al., 2020). Plastic pollution has been studied extensively in the ocean (e.g. Cózar et al., 2014, 2017). Less attention has been paid to its study in coastal and inland wetlands, although they can be the sinks for plastics spread from the surrounding land, especially in the case of wetlands lacking outflows (Nava et al., 2023; Morales-Caselles et al., 2021; Taylor et al., 2021).

Addressing the problem of plastic pollution requires an understanding of transport pathways and vectors. Physical drivers (i.e. wind, surface water runoff, sea currents) are usually considered to be the main carriers of plastic debris, and this is likely the case in most environments (Cózar et al., 2017; González-Fernández et al., 2021). However, the potential role of animals as natural vectors for plastic transport could be greater than expected in some environments (Bourdages et al., 2020; Ballejo et al., 2021). Biovectors can become highly significant because of their abundance and the non-random nature of their movements (e.g. birds feeding in one place then moving to another to roost), which can promote the accumulation of contaminants in particular sites (Blais et al., 2007). Studies of biovectoring of contaminants by birds are relatively scarce and have focused mainly on nutrients

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(“guanotrophication”: Fujita and Kameda, 2016; Martín-Vélez et al., 2019). Despite the fact that plastic ingestion has been reported in a wide range of avian species, such as raptors (Zhao et al., 2016; Ballejo et al., 2021), seabirds (Seif et al., 2018), waterbirds (Holland et al., 2016; Nicastro et al., 2018; Liu et al., 2023) and passerines (Zhao et al., 2016; Deoniziak et al., 2022), most research has merely highlighted the presence of plastic, or focused on the physiological and mechanical impacts within the bird digestive tract (Ghaffar et al., 2022; Roman et al., 2022). There remains limited understanding of the biovectoring role of birds in the transport of plastic debris into natural ecosystems (Bourdages et al., 2020) and particularly into wetlands, yet movement ecology studies suggest it may be important (Martín-Vélez et al., 2020; López-Calderón et al., 2023).

A wide variety of opportunistic species feed in landfills, where food is available all year-round (Plaza and Lambertucci, 2017; Seif et al., 2018; Arnold et al., 2021). This phenomenon may have important consequences for the population dynamics and migratory behavior of such species, as is the case for the white stork *Ciconia ciconia* (Tortosa et al., 2002; Kruszyk and Ciach, 2010; Flack et al., 2016; Arizaga et al., 2018; Bécarea et al., 2019). White storks frequently feed in landfills as a time and energy-saving foraging strategy (Soriano-Redondo et al., 2021; Marcelino et al., 2023). Consequently, they have modified stopover and wintering areas, reduced their migration distances, and even become sedentary in areas close to landfills. All these changes have driven higher survival, reproductive success and population sizes (Flack et al., 2016; Gilbert et al., 2016; Arizaga et al., 2018; Cheng et al., 2019; Soriano-Redondo et al., 2023). White storks have become common landfill

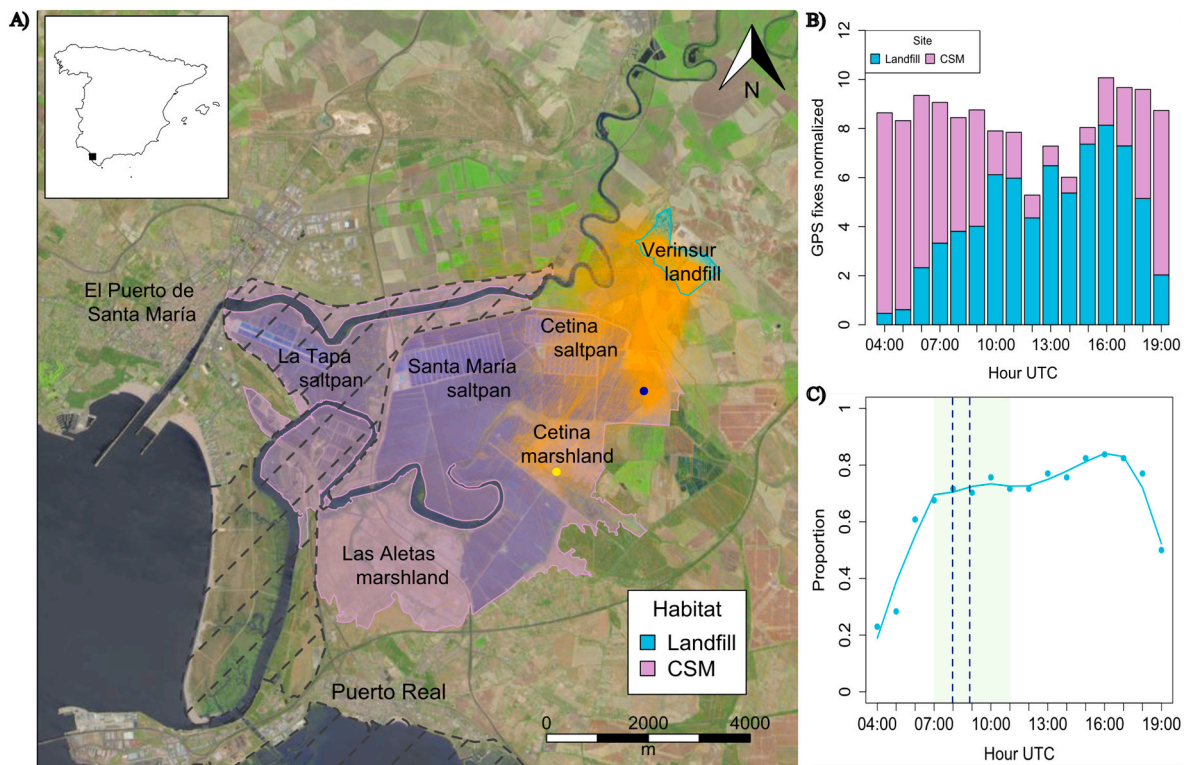
“clients” where they ingest food, but also contaminants such as plastics (Peris, 2003; Henry et al., 2011; Nicastro et al., 2018). White storks constitute one of the best models to study plastic transport because, after feeding at dump sites, they frequently move to wetlands for roosting. In addition, there are many storks tagged with GPS devices to track their movements (e.g. Flack et al., 2016; Blas et al., 2020; López-Calderón et al., 2023).

Here, we provide the first study to quantify the role of white storks in the movement of plastics from landfills to wetlands. We focused in a “Complex of Salt ponds and Marshes” (CSM) located in the Bay of Cádiz (Spain), which is used as a major stopover site on the flyway between Europe and Africa. This CSM is used by concentrations of migratory storks foraging in a nearby landfill. First, we quantified the amount and nature of plastics egested by white stork in the CSM, by investigating regurgitated pellets. Second, we estimated the numbers of storks that roost in the CSM each day using both GPS and field census data. Finally, this information was used to estimate daily plastic loading by storks into the CSM, and to identify contamination hotspots using GPS information.

## 2. Material and methods

### 2.1. Study area

Our study was carried out in the Cadiz Bay area of Andalusia, southwest Spain (36°35'N 6°08'W) close to the towns of Puerto Real and El Puerto de Santa María. This area is one of the most important for salt production in Spain, with a complex of intertidal mudflats and artificial



**Fig. 1.** A) Study area, constituted by Verinsur landfill and the complex of salt ponds and marshlands (CSM) used by white storks. Orange lines represent GPS tracks from one standing position to the next one by each individual registered in the area. Dashed polygons represent Cádiz Bay Natural Park. Dots show where pellets were sampled (dark blue in September 2021, yellow in January 2022). Image downloaded from Sentinel-2, at: <https://earthexplorer.usgs.gov>. Satellite image is from July 10th 2022. Map generated with raster (Hijmans, 2022) and sp. (Pebesma and Bivand, 2005) packages in R (R Core Team, 2022). B) Sum of GPS fixes normalized by individuals detected in CSM and the landfill (i.e. a fix for each bird-year counts as 1/total number of fixes registered for that bird-year). Data were obtained by 74 bird-years in each location between 2013 and 2022. C) Proportion of all tagged bird-years registered in the landfill for each hourly interval (data points representing 04:00–04:59 h, etc.). Points and smoothed line represent the observed proportions and LOESS predictions respectively. Blue-dashed lines and the green rectangle represent the mean start/end times of censuses and the period between the earliest start and the latest end time respectively. In Spain, local time is UTC+1 in winter and UTC+2 in summer. Hours between 19:00–04:00, when GPS devices were programmed to switch off, are not represented. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

solar saltworks including Santa María, La Tapa and Cetina, as well as adjacent marshes (Camilleri, 2015). All these salt ponds and marshes (included in our CSM) are also listed as important foraging and roosting sites for migrant birds (Infante et al., 2011; Bécarea et al., 2019; Martín-Vélez et al., 2022; López-Calderón et al., 2023). Part of this area is protected by the Ramsar convention and the EU Birds and Habitats Directives. The study area includes an urban landfill (Verinsur, Fig. 1A), which is a major feeding site for gulls (Martín-Vélez et al., 2021) and white storks (López-Calderón et al., 2023). After feeding bouts, white storks systematically move from the landfill to roost at the CSM, 2 km away (Fig. 1A–B).

## 2.2. Field sampling and sample processing

Analysis of regurgitated pellets is a standard method to study diet of waterbirds and raptors (Peris, 2003; Sánchez et al., 2005; Rosin and Kwiecinski, 2011) and to study contaminant egestion by scavenging birds (Provencher et al., 2019). Pellets are readily collected from spots where they concentrate to roost (Martín-Vélez et al., 2022). In order to quantify the plastic egestion by white storks, we collected 42 pellets (Fig. S1A) at the salt ponds on 27th September 2021 (22, from dykes close to evaporation ponds where salt crystals are extracted) and 21st January 2022 (20, from adjacent marshes, Fig. 1A). Pellets were collected at the peaks of migratory passage, i.e. two periods when the largest concentrations of storks can be found in the study area. All samples were then frozen ( $-26^{\circ}\text{C}$ ) until processing. Faecal samples were also collected and processed in a similar way, but their plastic content was trivial in comparison with the pellets (see Section 2.6). Pellets were dried at  $50^{\circ}\text{C}$  during 3 h, and then placed in a glass desiccator for another 3 h before measuring the dry weight (to the nearest 0.0001 g, on a Voyager Pro OHAUS VP214C balance). Pellets were then rehydrated with tap water and their contents were sieved through a 0.5 mm mesh. The remaining sieved material was placed on petri dishes and inspected through a stereomicroscope. The operator picked out and classified debris into three different categories: Highly probable plastic (HPP), possible plastic (PP), and other debris. Plastic items were identified by visual inspection as synthetic material difficult to break, generally waterproof, with straight borders and of low density. Items were categorized as PP when there was doubt about their synthetic origin because some of these characteristics were not fulfilled. Each debris category was then weighed separately and photographed with a NikonD3500 camera, using white paper as the background and graph paper for scale (Fig. S1).

## 2.3. Fourier transform infrared (FTIR) analysis

To identify the kind of plastics found in our samples, we selected representative items from each pellet that differed in texture and colour for both categories, HPP and PP, including at least one item per pellet when possible. Only items larger than  $4\text{ mm}^2$  were analysed by FTIR spectroscopy, as required by the Attenuated Total Reflectance Technique equipment (INVENIO X FTIR Research Spectrometry). A total of 187 items were identified by FTIR (158 for HPP, and 29 for PP). We used a multichannel analyser performing 32 spectra in wavelength ranges from  $400$  to  $4000\text{ cm}^{-1}$  to get raw IR spectra for each debris item. Standard blank measurements were taken through air every 10 samples, and the spectrometer surface was cleaned with 70 % ethanol after measuring each item. After obtaining raw spectra, we proceeded to their treatment (i.e. correction and smoothing) using the package *OpenSpecy* in R. We followed guidelines described in Cowger et al. (2021), and used function parameters that provided the best fit to our items. When a comparison with the corrected and smoothed spectra provided a hit quality index  $>800$ , a given item was assumed to have the corresponding identity. If the result obtained was not congruent to the analysed item (e.g. because we obtained an identity “beeswax” for an item that was “clearly a film plastic”), we chose the next congruent result

with a similar spectrum, provided it had a hit quality index  $>800$ . After having assigned each item to a specific type of material, these were classified as plastic or no plastic following Hartmann et al. (2019).

Based on the FTIR results, we corrected the initial weights obtained for plastics and other debris. For the HPP category, 66 % of items were identified as plastics, compared to 31 % for PP. Therefore, the weights obtained in each pellet for HPP and PP were multiplied by 0.66 and 0.31 respectively, to estimate the total plastic weight in each sample. Similarly, we multiplied the weight of HPP and PP by the fraction of items recognised as non-synthetic debris in HPP and PP (0.11 and 0.34 respectively), to obtain the verified total weight of other debris in each pellet, after summing with the initial other debris category. 23 % of items identified were natural material in HPP and 35 % in PP (e.g. arthropod exoskeletons or leaves), which were therefore excluded from our calculations of biovectoring of anthropogenic debris.

## 2.4. GPS data on stork movements within our study area

We analysed GPS movements of white storks downloaded from Movebank (see Data availability section). Specifically, we used a combination of storks breeding in Germany (tagged during 2013–2014; Cheng et al., 2019) and Spain (tagged during 2013–2015; Blas et al., 2020). All individuals were tagged with GPS-ACC loggers (e-obs GmbH; Munich, Germany), attached as a backpack with a Teflon-nylon harness (for details see Cheng et al., 2019 and Blas et al., 2020). GPS devices were programmed to save one position each 5 min (when possible, depending on battery load and GSM network). We filtered GPS fixes on land in Cadiz province ( $36^{\circ}$ – $36.62^{\circ}\text{N}$ ,  $6.3^{\circ}$ – $5.6^{\circ}\text{W}$ ), using only non-flying fixes as defined by velocity  $< 10\text{ km/h}$  (Van Coppenolle and Aerts, 2004; López-Calderón et al., 2023). We used GPS data to identify foraging and roosting sites by looking for hotspots of stork activity, and found a clear pattern of movements between CSM and the landfill. We made a new filter using only non-flying individuals in the CSM polygon (area  $38.23\text{ km}^2$ ) and the landfill polygon (area =  $1.07\text{ km}^2$ ). A total of 56 different individuals were finally filtered for this study (Table S1), 46 individuals breeding in Germany and 10 breeding in Spain. Some individuals in this GPS dataset were present in our study area across different years, so we defined “bird-year” as the consecutive positions of a given individual from 1st June in a given calendar year to 31st May the following year, following the annual life cycle of migratory storks passing through the study area (Bécarea et al., 2019). For example, the set of GPS fixes of the stork tagged as “3027” from June to December 2014 is the bird-year “3027W2014”; and the set of GPS fixes of this stork from January to May 2015 is also the same bird-year. Hence, we considered bird-year as the unit of measurement within our GPS dataset. As shown by López-Calderón et al. (2023), our study area (CSM) is a major sink for stork flights coming from landfills, resulting in a total of 79 bird-years that mostly forage in the landfill during the days and roost in CSM during the night (Fig. 1B).

We also used GPS data to quantify seasonal differences in use of the study area. For this purpose, we calculated the number of bird-years that roosted at the salt ponds and adjacent marshes for each ordinal date by filtering the GPS fixes over the CSM polygon (boundaries shown in Fig. 1A). This filter resulted in 74 bird-years, with 53 different individuals, from 2013 to 2022. In other words, we pooled together all years in the GPS dataset to quantify the seasonal use of CSM as measured by the number of different bird-years summed for each ordinal date. Given the variability from one day to the next, we then calculated the predicted number of bird-years by fitting LOESS (Locally Estimated Scatterplot Smoothing, with span parameter 0.45) to the raw number of bird-years for each ordinal date. LOESS is a local polynomial regression that smooths dataset trends based on Least Squares methods (Gijbels and Prosdoci, 2010). Details of number of days and fixes spent by each bird in CSM and the landfill are in Table S1.

### 2.5. Census data

Regular censuses were performed in 2022 ( $n = 24$ ), approximately twice a month at the landfill during the morning between 7:00 h and 11:00 h UTC. All stork individuals on land were counted. To confirm that the number of storks that rest on CSM each day is almost the same as the number of storks foraging at this landfill (Fig. 1B), we filtered GPS fixes over Verinsur landfill polygon (Fig. 1A), resulting in 74 bird-years. We then performed a linear regression with the number of bird-years in the landfill on each ordinal date as the predictor variable, and the corresponding bird-years in CSM as the dependent variable. The number of bird-years in Verinsur explained most of the variation in number of bird-years present in CSM ( $R^2 = 83.9\%$ ), the intercept did not significantly differ from zero (estimate = 0.16; SE = 0.13;  $p = 0.22$ ) and the slope of the regression was close to one (estimate = 0.91; SE = 0.02;  $p < 0.0001$ ).

Census counts were corrected using GPS data to obtain the best estimate for the total number of birds present in the landfill on a given day. We divided the period 4:00–19:00 UTC (earliest and latest hours with GPS fixes at the site) into 1-hour intervals, and calculated the number of bird-years in each hourly interval across all GPS fixes within the landfill. We then divided the number of bird-years that has ever been registered in each hourly interval by the total accumulated number of bird-years that visited the landfill (i.e. 74), thus obtaining the proportion of storks present for that interval. Then, a LOESS (span 0.45) was fitted for the proportion of storks present in response to the hourly interval (Fig. 1C). Finally, we divided raw census counts by the mean LOESS-predicted value for the hourly periods covered by a given census, to correct for birds missed by the census (i.e. those visiting the landfill at different times of the day). In order to estimate the number of white storks using CSM as roosting site every day, we assumed that all birds visiting the landfill (quantified as above) roosted in CSM. We fitted a LOESS function (span 0.45) for the corrected census counts in response to ordinal dates. Details of raw and corrected census are presented in Table S2.

### 2.6. Plastic loading model

Daily Plastic Loading (DPL) by white storks into CSM was estimated as follows:

$$DPL = \sum_{i=1}^{DNB} ER * RPW_i,$$

where DNB = Daily Number of Birds in 2022, estimated by the above LOESS-predictions for corrected censuses in the landfill; RPW = Random Plastic Weight selected from the estimated plastic weights contained in the 42 pellets processed in the lab, corrected after FTIR; ER = Egestion Rate for pellets. In field conditions, male white storks egest on average 1.5 pellets per day and females one per day, generally at night (Zbigniew Kwieciński, unpublished data). We made the conservative assumption that a given white stork of either sex regurgitates one pellet per day in CSM.

We focused on plastic loading within pellets, because they include the overwhelming majority of the mass of plastics  $\geq 0.5$  mm transported by storks. Our preliminary analysis of faecal samples from CSM suggested that mean daily mass of plastics ( $\geq 0.5$  mm) moved by storks in faeces was  $< 1\%$  of that moved in pellets. Furthermore, the small size of fragments found in faeces made it impractical to study them with our FTIR methodology (which requires items to have  $\geq 4\text{mm}^2$ ). We estimated DPL for each ordinal date of 2022, allowing us to explore seasonal differences in plastic loading to CSM. Because our model involves randomizations (with resampling), we repeated DPL estimations 10,000 times, in order to provide measures of uncertainty (e.g. range across simulations). We also performed a similar model to quantify the numbers of plastic particles of  $\geq 0.5$  mm transported to CSM in stork pellets during 2022.

Finally, we elaborated a heatmap to estimate spatial plastic deposition in CSM. We standardized the difference in time between GPS fixes previously filtered over CSM, eliminating those that differ  $> 6$  min or  $< 4$  min from previous fixes. We then built a Kernel Utilization Distribution function for the fixes, which provides the probability to find a stork in a given pixel. We used the function kernelUD from the library *adehabitatHR* (Calenge, 2006) with grid = 1500, h = hrfe and extent = 0.43 to obtain a raster layer with 1,744,500 pixels of 10 m size, and we masked it using the CSM polygon. To estimate the plastic loaded to each pixel, the probability of finding a stork in a given pixel was normalized to the sum (i.e. the sum of all KUD pixels had the value of 1) and multiplied by the total weight of plastic transported during 2022 (i.e. the sum of DPL). In this way, the sum across all pixels of the heatmap equals our estimation of total plastic loaded to CSM in 2022. This approach assumes that stork locations (when not flying) are good predictors of where pellets are egested, which is supported by our experience when collecting pellets (see also Martín-Vélez et al., 2022).

## 3. Results

### 3.1. Plastic and other debris content

Total dry weight of stork pellets ( $N = 42$ ) ranged from 3.52 to 33.98 g (mean = 10.73 g; SD = 6.74 g). Almost all pellet samples (41 of 42) contained plastic, with a mean weight (estimated after correction with FTIR) of 0.47 g per pellet (range: 0–3.67 g; Fig. 2). The only pellet without plastics contained other anthropogenic debris. There were no significant differences in plastic presence (binomial-GLM estimate = 18.55 SE = 4924.77;  $p = 0.997$ ) or plastic mass (gamma-GLM estimate = -0.45; SE = 0.99;  $p = 0.65$ ) between pellets collected in different months (September 2021 vs January 2022). Other debris in pellets, corrected after FTIR, had a mean weight of 0.67 g (range: 0–9.81 g). Plastic particles ranged from 0 to 32 particles per pellet, with a mean of 9.64. A mean of 14 non-synthetic anthropogenic particles were found in each pellet (range: 0–36) (Fig. 2).

The distribution of particle sizes revealed a smaller proportion of

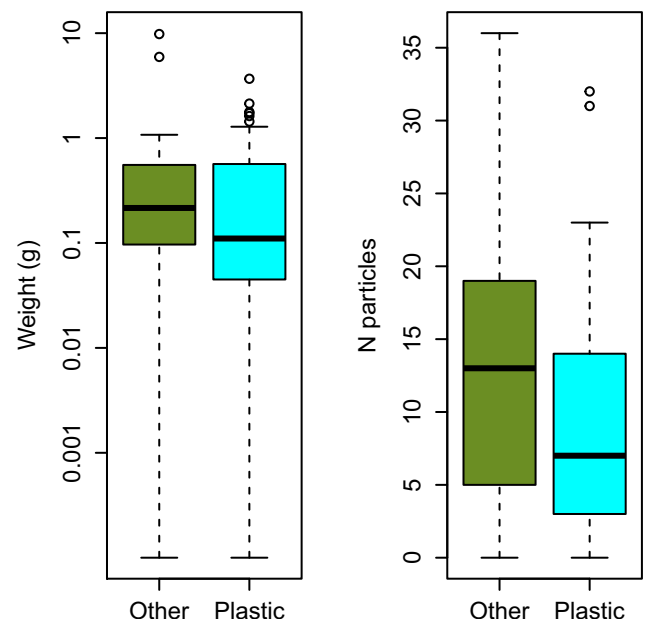


Fig. 2. Boxplots of weight and number of particles of plastic and other debris extracted from stork pellets. Left graphic represented in a logarithmic scale (0.0001 was summed to all values). Upper margin, middle line, lower margin and bars outside the box represent the third, second and first quartiles and the minimum and maximum values respectively. Outliers exceeding the upper  $1.5 * \text{Inter-Quartile-Range}$  are represented as dots.

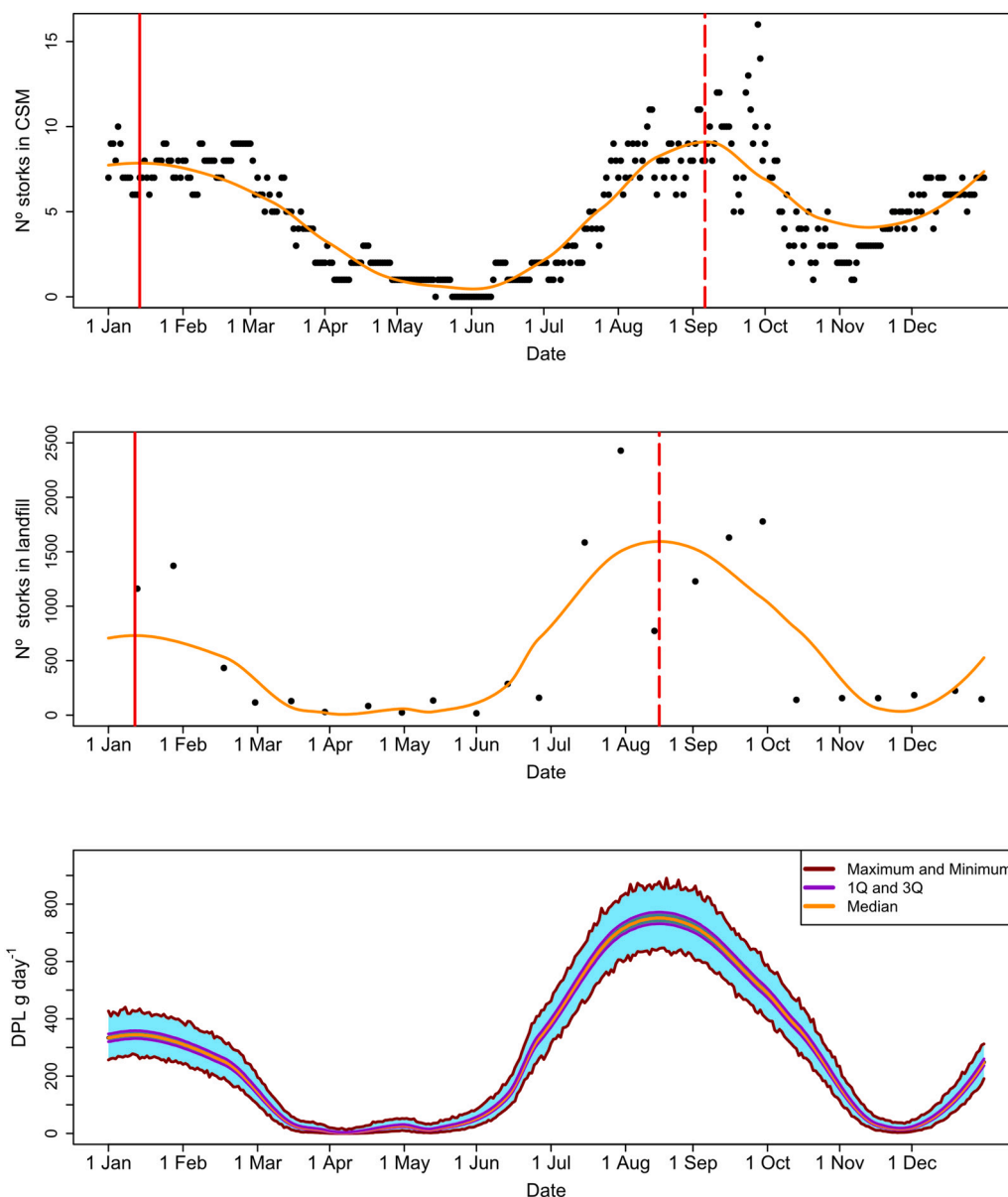
macroplastics (>2 cm, 7.97 %), followed by mesoplastics (from 5 mm to 2 cm, 40.58 %), with microplastics (<5 mm) the most common particle size (51.45 %), even though items under 0.5 mm were not retained in our sieve. Plastics identified were mostly polyethylene (21.05 % of items, including Low-Density and High-Density polyethylene), silicone (16.67 %), polypropylene (14.91 %) or polystyrene (14.04 %) (Table S3). Among other debris, the most common material was glass, but aluminium, other metals, cellulose derivatives and non-synthetic textiles were also found (Table S4).

### 3.2. Temporal use of the study area by white storks

GPS tracks showed a clear pattern of daily movements from the CSM to the landfill at dawn, and from the landfill to the CSM around dusk (Fig. 1A–B). Moreover, GPS data showed that both sites together constitute a stopover for Western European migratory storks. Considering the life cycle of GPS-tagged storks (Fig. 3A), we defined a first

migration period from June to November (southbound/autumn migration) and a second one from December to May (northbound/spring migration). Specifically, the LOESS fitted to GPS data indicated that these migration periods started on 2nd June and 13th November (based on minimum numbers of predicted bird-years), with peaks on 6th September and 14th January. A total of 61 different bird-years stayed in CSM during the autumn migration (from 2nd June to 13th November) for 14 days on average, whereas 25 bird-years stayed in CSM during the spring migration (from 14th November to 1st June) for 38 days on average.

Seasonal patterns in census data (2022) generally overlapped with those from GPS data (2013–2022). For instance, peaks in seasonal use by storks based on census data (18th August for autumn and 12th January for spring migration; Fig. 3B) were fairly close in time to those given above for GPS data.



**Fig. 3.** A) Number of bird-years for daily white storks roosting in CSM (pooling all years in the GPS dataset). Orange lines indicate LOESS predictions. Continuous and dashed lines represent dates of spring and autumn migration maxima (in red) respectively, obtained from estimated values (also for fig. B). B) Storks counted during each landfill census after correction (see Section 2.5). C) Daily Plastic Loading by white storks in CSM estimated for 2022, simulated 10,000 times. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

### 3.3. Plastic loading and other debris simulations

Our plastic loading model simulated daily plastic deposition by white storks during 2022 (Fig. 3C). We then calculated “Yearly Plastic Loading” (YPL) as the accumulated plastic loading for 2022 in a given simulation (i.e. one out of 10,000). The mean YPL calculated across the 10,000 simulations was 99.49 kg (range: 98.16–100.82). DPL showed a broad range of values from the minimum to the maximum estimate for a given day in our simulations (Fig. 3C); e.g. for 18th August the range was 645.14–878.67 g day<sup>-1</sup>; for 8th April it was 0.11–18.25 g day<sup>-1</sup>). This seasonal pattern is a consequence of both the number of storks in the field and the distribution of plastic weights within pellets, since many had plastic weights close to zero whereas a few had very high values (Fig. 2). CSM covers 38.23km<sup>2</sup>, so the mean loading of plastics was 2.60 kg km<sup>-2</sup> year<sup>-1</sup>. However, plastic contamination is not homogeneously deposited across the salt ponds and marshes, and there were hotspots of pollution where storks congregate to rest (Fig. 4).

We performed similar simulations randomizing the number of plastic particles counted in each pellet, and the other debris weight. Thus, the estimated plastic particle loading was 2,033,823 particles year<sup>-1</sup> (range: 2,020,902–2,047,306) divided into 162,096 macroplastic (7.97 %), 825,325 mesoplastic (40.58 %) and 1,046,402 microplastic (51.45 %) particles (the latter being an underestimate, since we ignored particles of <0.5 mm). The total amount of other anthropogenic debris loading in CSM was estimated at 141 kg year<sup>-1</sup> (range: 137.94–143.64).

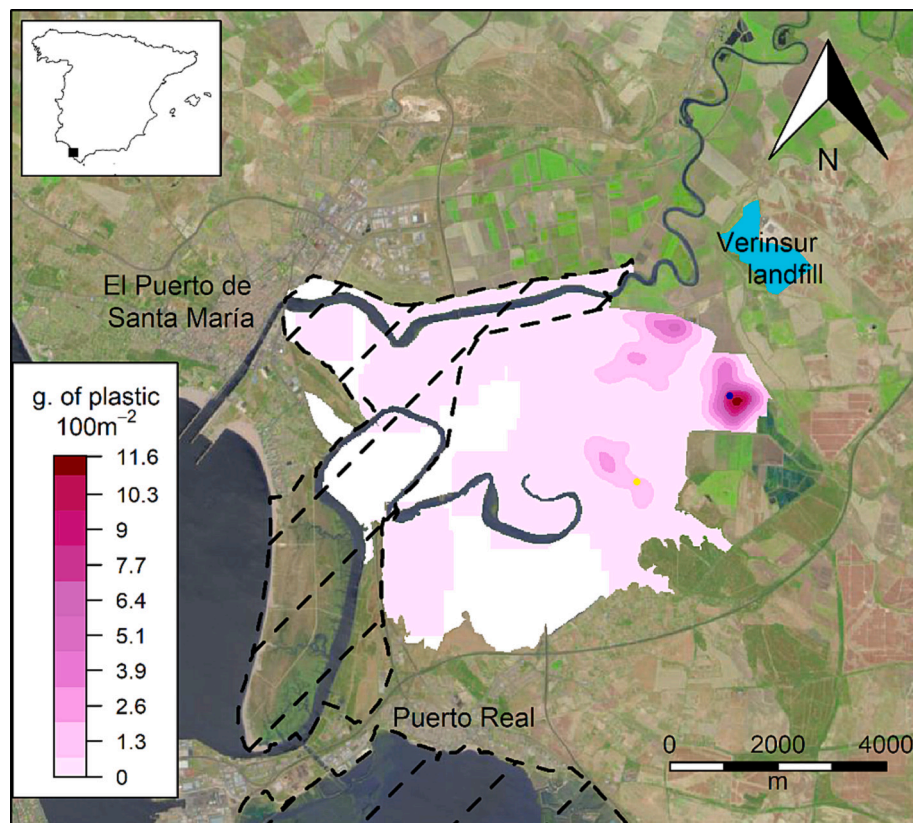
## 4. Discussion

Our study demonstrates the role white storks can play as biological

vectors of plastics from landfills into wetlands important for biodiversity. We identified specific sites where storks feed and then egest plastics, estimating the total loading of plastics during an annual cycle. As far as we know, this is the first study to quantify plastic loading by birds from a landfill into a coastal wetland. Plastic pollution research is largely focused on abiotic vectors (Castro-Jiménez et al., 2019; Schmid et al., 2021), and our work provides an important advance to our understanding of plastic contamination in coastal water bodies by biovectors (Holland et al., 2016; Bletter and Wantzen, 2019). The mean loading of plastics we calculated of 2.60 kg km<sup>-2</sup> year<sup>-1</sup> in the CSM is equivalent to >500 plastic shopping bags km<sup>-2</sup> year<sup>-1</sup>. As a reference, estimates of atmospheric inputs of plastic transported by wind and rain into protected areas of the USA were of 2.0–7.6 kg km<sup>-2</sup> y<sup>-1</sup> (Brahney et al., 2020).

### 4.1. Kinds of plastic carried by storks, and spectroscopy techniques

The separation and identification of plastics can be unreliable, especially for inexperienced researchers (Xu et al., 2019). Spectroscopy techniques, such as FTIR, are important to avoid overestimating plastic content in pellets. However, some items categorized as not plastics by FTIR may be plastics misidentified due to solar and chemical degradation, which can affect FTIR spectra (Xu et al., 2019; Dimassi et al., 2023). The observed distribution of plastics is consistent with previous research, since polyethylene and polypropylene are among the most common plastic debris in gull pellets (Almeida et al., 2023) and ecosystems (Rowley et al., 2020). Polypropylene and polyethylene are also the two main plastics produced in Europe and are dominant in the packaging industry (PlasticsEurope, 2022). We also found silicone to be



**Fig. 4.** Heatmap of plastic deposition during 2022 on CSM (complex of salt ponds and marshes). The total probability of finding a white stork across the Kernel Utilization Distribution was normalized to 1 and multiplied by the total amount of plastic deposited by storks in 2022 (99.49 kg). The heatmap was masked by CSM boundaries, representing where birds roost (in dark violet) and places where no plastic deposition is predicted (in white). Shaded polygons represent Cádiz Bay Natural Park. Dots show where pellets were sampled (dark blue in September, yellow in January). 11.6 g 100 m<sup>-2</sup> are equivalent to 1.16 kg ha<sup>-1</sup>. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

common, perhaps because it is used to make rubber bands, which are frequently ingested by storks (Henry et al., 2011; Fig. S2).

Our estimates of plastic loading by storks were conservative, since we excluded plastic fragments of <0.5 mm which are likely to be abundant in faeces. Larger, resistant particles ingested by waterbirds tend to be retained in the gizzard and expelled within pellets, whereas the smallest particles are likely to enter the intestines and be expelled in faeces (Sánchez et al., 2005; Provencher et al., 2018a). Estimated numbers of plastic particles moved into salt ponds and marshes would be higher if the cut-off was reduced below 0.5 mm. Our focus on pellets and larger particles is justified since our main interest was the mass of plastics transported, and tiny particles (<0.5 mm) would represent a negligible fraction of total mass. Ultimately, large particles deposited in stork pellets will break up into many smaller particles (Cózar et al., 2014; Andrady, 2017).

#### 4.2. From studies of plastic ingestion to plastic loading

Most previous studies on plastic consumption by birds focused on digestive tract analysis (Henry et al., 2011; Zhao et al., 2016; Holland et al., 2016; Seif et al., 2018) or quantified plastics in pellets (e.g. of vultures that feed in landfills, Ballejo et al., 2021), but did not quantify plastic loading into the environment. Previous work on storks has studied plastics inside birds found dead or severely injured (Peris, 2003; Nicastro et al., 2018), but this is inherently biased since these birds are particularly likely to have ingested large plastic items. Nicastro et al. (2018) found polyethylene, silicone and polystyrene as the most prevalent type of plastic eaten by storks, but polypropylene that did not appear in their results. A few studies have quantified the active transport and deposition of plastic by seabirds in the marine environment. Bourdages et al. (2020) estimated that fulmars (*Fulmarus glacialis*) and murrelets (*Uria lomvia*) transported 3.3 and 45.5 million plastic particles each year respectively within their breeding colonies. Similarly, Grant et al. (2021) estimated that flesh-footed shearwaters (*Ardenna carneipes*) transported 165 kg of plastic per year from the ocean to their breeding colony on an oceanic island.

GPS data provide a key tool to understand the role of birds in contaminant flux. It facilitates the identification of foraging and roosting sites, detecting potential pollution hotspots caused by birds over broad geographical scales (Martín-Vélez et al., 2020; López-Calderón et al., 2023). In the absence of movement data, waterbird censuses have been used to estimate nutrient loading into wetlands (e.g. Hahn et al., 2007; Hahn et al., 2008; Winton and River, 2017). However, as well as being vital to identify daily movements from landfills to roosting habitats, we have shown how GPS data allows the correction of censuses for birds missing at the time of counting, which further improved our estimates of plastic loading.

Nevertheless, there were limited seasonal differences between GPS and census estimations. Notably, juvenile white storks are more likely to die on their autumn migration than adults (Schaub et al., 2005; Cheng et al., 2019), and thus many more juveniles are counted in the field during autumn than during spring migration. However, this difference was reduced in our GPS dataset. Additionally, inter-annual differences may also explain why seasonal use in 2022 based on census data is not identical to that for the period 2013–2022 based on GPS data (Fig. 3A–B).

#### 4.3. Wider implications for biovectoring by storks and other waterbirds

Our study provides unique census estimates and confirms that CSM is an important stopover site for white storks migrating between Spain and Morocco (Bécares et al., 2019; López-Calderón et al., 2023). The last national census of the white stork population in Spain estimated 36,217–37,556 individuals during the 2020 winter, an increase of 5000 individuals since 2004 (SEO/BirdLife, 2020). The wintering population which stays in Southern Europe instead of proceeding to Africa has

grown by 121 % between 1997 and 2017 (Bécares et al., 2019), due largely to the food sources provided by landfills (Flack et al., 2016). This suggests that our study is relevant across the whole range of white storks, where plastic ingestion is widespread, and the quantity of plastic transported by storks to wetlands and other habitats has likely increased in recent decades. It is likely that the plastic loading identified in our study is a small proportion of that occurring through storks across Spain (see López-Calderón et al., 2023 for the importance of other landfills to migrating storks). In addition to white storks, there are other bird species feeding at the landfill in our study area that are likely contributing to plastic accumulation, notably the lesser black-backed gull (*Larus fuscus*; Martín-Vélez et al., 2021) and the yellow-legged gull (*Larus michahellis*). The relative importance of these gulls and storks for plastic loading in Cadiz Bay is worthy of future investigation.

The effects of ingested plastics on birds and other animals can come from internal physical damage (Henry et al., 2011, Fig. S2), satiation with debris and thus starvation (Holland et al., 2016; Wright et al., 2013; Zhao et al., 2016), and due to toxic additives. For example, flame retardants can accumulate in bird tissues in response to plastic consumption (Provencher et al., 2018b; Cheng et al., 2020) or cause fibrotic disease in birds known as ‘Plasticosis’ (Charlton-Howard et al., 2023). After plastic particles are released into wetlands or other habitats in pellets or faeces, they can lead to a wider range of environmental impacts (Wright et al., 2013; Sun et al., 2022); for example, the transference of hydrophobic pollutants to the environment (González-Soto et al., 2019). As particle size reduces, plastics can enter the food web (Hammer et al., 2015; Zalasiewicz et al., 2016; Sendra et al., 2020) and accumulate in phytoplankton and aquatic invertebrates, such as *Artemia franciscana* (Sendra et al., 2020) which is abundant in CSM and ingested by other waterbird species that do not feed in landfills (Sánchez et al., 2006; Varo et al., 2011). Plastics can also adsorb toxic compounds (Teuten et al., 2009), act as a reservoir of bacteria biofilms and antibiotic resistant genes (Piergiacomo et al., 2022; Liang et al., 2023), and facilitate transference of antimicrobial resistant genes (Yuan et al., 2022).

Our heatmap identified hotspots of contamination in transformed marshes that nowadays depend on rainfall, including one next to a solar salt pond. These areas lack an outflow to the ocean and water entering is lost by evaporation. Consequently, plastic will likely accumulate in these areas, either in sediments or in food webs (Zalasiewicz et al., 2016). Together with water flow, wind is a major abiotic vector for plastics (Cózar et al., 2017; González-Fernández et al., 2021) and plastics deposited in stork pellets may later be blown by strong prevalent winds into salt ponds (Fig. 1A). Nevertheless, salt products worldwide contain microplastics, caused mostly by contamination of marine water supplying salt ponds (Lee et al., 2019; Zhang et al., 2020).

#### 4.4. Implications for management

To solve the environmental problems related to biovectoring by waterbirds, better waste management (e.g. reducing the availability of organic waste) would reduce the visits of scavenging birds by limiting access to food (Arévalo-Ayala et al., 2023). The closing of open landfills should also be an effective measure (Langley et al., 2021), and could also reduce greenhouse gas emissions (Limoli et al., 2019). Alternatively, some projects have tried to tackle problems derived from bird scavenging on landfills using distress calls, pyrotechnics, shooting or visual signals (Baxter and Robinson, 2007; Soldatini et al., 2008; Baxter and Allan, 2010; Castège et al., 2015). No one has demonstrated clear long-term effects, but a combination of techniques appears to be more effective (Baxter and Robinson, 2007; Soldatini et al., 2008; Baxter and Allan, 2010; Castège et al., 2015), although no studies have focused on stork deterrence. Periodical cleaning operations in hotspots of plastic loading identified by GPS, especially following migration events, could help reduce local accumulation of plastics in the CSM.

## 5. Conclusions

Wind, surface runoff or marine currents are the vectors traditionally considered in the analysis of plastic transport and distribution (e.g. Cózar et al., 2017; González-Fernández et al., 2021). We focus on plastic transport by white storks from a landfill into wetlands. For the first time, the order of magnitude of plastic loading by waterbirds from a landfill to wetlands has been quantified. The combination of census data, GPS data, pellet analysis and FTIR technology allowed the development of a reliable dispersal biovectoring model to estimate daily variations of plastic transport. Our methods could be applied in future studies elsewhere, especially given abundant movement information now available on storks and other waterbirds. The plastic loading of transported to CSM was estimated to be around 100 kg year<sup>-1</sup>, with a mean density of 0.026 kg ha<sup>-1</sup> year<sup>-1</sup>, and local concentrations exceeding 1.2 kg ha<sup>-1</sup> year<sup>-1</sup> in the area close to salt extraction ponds. Our study reveals the importance of waterbirds in the movement of plastics and other debris into wetlands.

## CRedit authorship contribution statement

Conceptualization: JCP, CLC, MIS, AC, MR, FH and AJG; Methodology: JCP, CLC, AJG, AC; Computation: JCP, CLC; Formal analysis: JCP, CLC, VMV; Data collection: JCP, FH, BCJ; Data curation: JCP, CLC, BCJ; Writing the initial draft: JCP; Critical review, commentary or revision: JCP, CLC, VMV, MIS, AC, MR, FH and AJG; Visualization: JCP; Supervision: AJG, MIS, CLC; Project administration: AJG; Funding acquisition: AJG, FH. All authors read and approved the final manuscript.

## 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

The datasets analysed during the current study are available in Movebank Data Repository, <https://www.movebank.org>. Specifically, five Movebank studies were used: “LifeTrack White Stork Oberschwaben” (doi:<https://doi.org/10.5441/001/1.c42j3js7>), “LifeTrack White Stork Bavaria” (doi:<https://doi.org/10.5441/001/1.v1cs4nn0>), “LifeTrack White Stork SW Germany” (doi:<https://doi.org/10.5441/001/1.ck04mn78>) “LifeTrack White Stork Rheinland-Pfalz” (doi:<https://doi.org/10.5441/001/1.4192t2j4>) and “LifeTrack White Stork Spain Donana” (doi:<https://doi.org/10.5441/001/1.78152p3q>).

Other data will be made available by 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.2023.115773>.

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