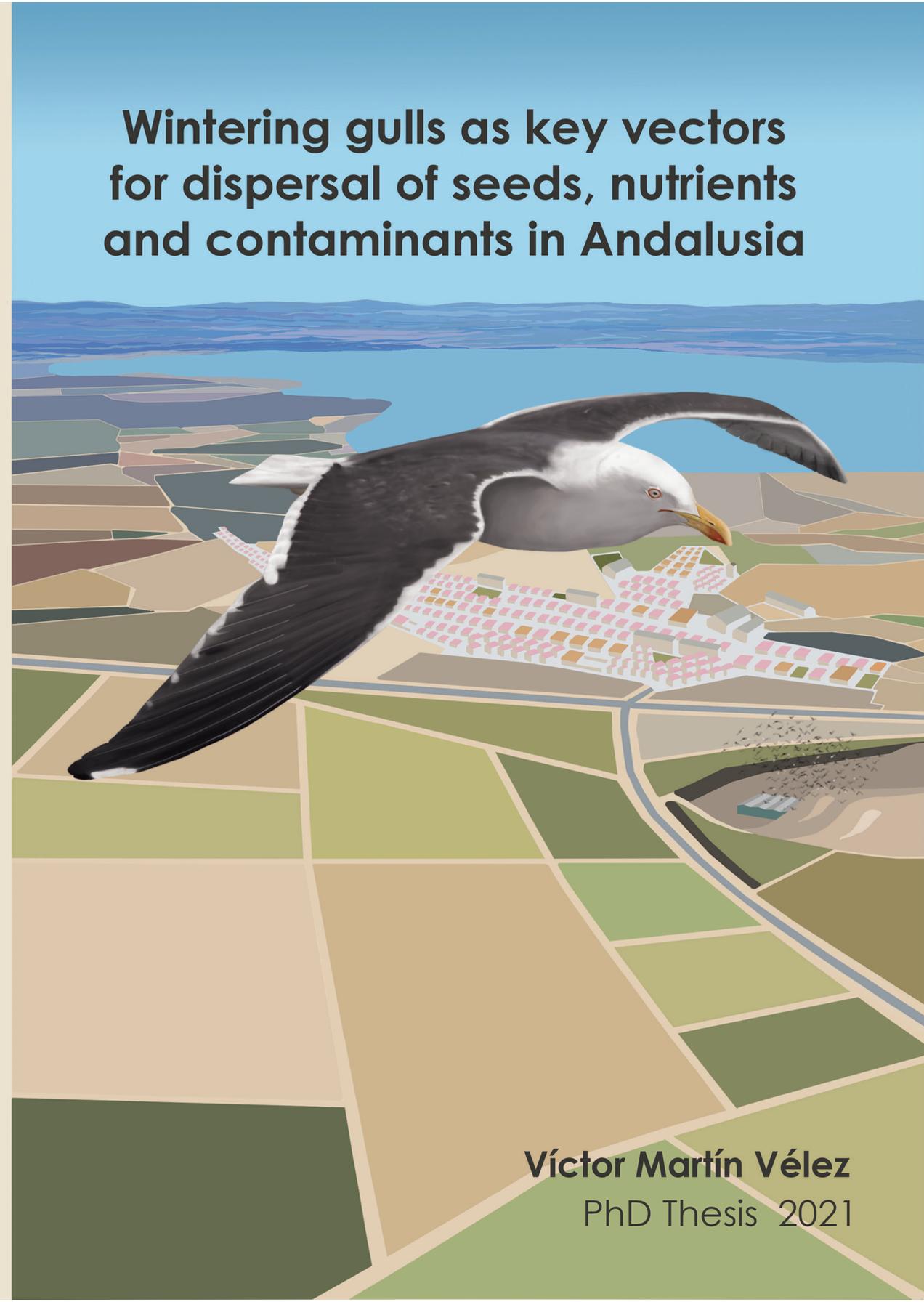




Víctor Martín Vélez
PhD Thesis 2021

Wintering gulls as key vectors for dispersal of seeds, nutrients and contaminants in Andalusia

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Universidad
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Ecosistemas

Wintering gulls as key vectors for dispersal of seeds, nutrients and contaminants in Andalusia

PhD Thesis

Víctor Martín Vélez

Thesis supervisors

Prof. Andy J. Green

Dr. Marta I. Sánchez

Tutor

Dr. Josabel Belliure

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*A mi madre y a mi abuela,
A mi familia,
A todes los que luchan día a día,*

*“La vida es una escalera, seguramente te caigas en algún escalón, pero
hay que levantarse y seguir subiendo”
- Mi abuela.*

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Resumen

Los servicios ecosistémicos de las aves acuáticas son diversos y procesos tales como la dispersión de semillas o el biotransporte se encuentran entre los más importantes. Debido al cambio global, muchos de estos servicios se pueden convertir en “diservicios” cuando las semillas dispersadas presentan una inconveniencia, como las malas hierbas, o el biotransporte de nutrientes y contaminantes ocurre a humedales, ya de por sí frágiles y vulnerables al cambio global. Los servicios de las aves acuáticas ya se han demostrado en estudios anteriores, pero la aparición de nuevas tecnologías GPS para el seguimiento de aves hace posible obtener movimientos detallados que hacen la cuantificación de esos servicios más realista.

En esta tesis doctoral, me centro en el papel de la gaviota sombría *Larus fuscus* en la dispersión de semillas y biotransporte a escala de Andalucía. La disponibilidad de datos GPS detallados de la invernada de esta especie, junto con su capacidad de explotar distintos hábitats, hacen que sea un buen modelo de estudio.

A través de la combinación de datos de movimiento GPS con análisis de redes, en el **Capítulo 1** se pudo generar una red de conectividad entre hábitats más utilizados por la gaviota sombría. De este modo, se puede determinar dónde y con qué frecuencia ocurren las conexiones entre hábitats más importantes y que, por lo tanto, están implicadas en los procesos de transporte de semillas y contaminantes. En este capítulo se muestra cómo los vertederos y los arrozales tienen un papel importante para mantener la conectividad entre hábitats generada por las gaviotas, sobre todo las conexiones que tienen como destino final los sistemas acuáticos de la región. La mayor parte de las conexiones entre hábitats se pueden agrupar en rangos de 60 km y dividir Andalucía en unidades funcionales. Esta sería la distancia donde los procesos de biotransporte y dispersión son más probables que ocurran.

El **Capítulo 2** está centrado únicamente en los arrozales de Doñana, donde se compara el potencial de dispersión de dos especies generalistas que se alimentan de la misma manera: la gaviota sombría y la cigüeña blanca. A través del estudio

Resumen

de excrementos y egagrópilas en el laboratorio y posteriores experimentos de germinación se mostró que la comunidad de plantas dispersadas por ambas especies es extensa abarcando 21 taxones diferentes, incluyendo malas hierbas, pero no hay variación de una especie a otra, pese a las diferencias en tamaño.

Sabiendo las especies de malas hierbas dispersadas, el **Capítulo 3** tiene como objetivo combinar datos de movimiento GPS de gaviota sombría con tiempos de retención de semillas (obtenidos de manera teórica y a través de experimentos en cautividad) para generar modelos espaciales de dispersión de semillas cuyo origen son los arrozales de Doñana. Debido a su extensión, gran cantidad de las semillas son depositadas dentro del mismo arrozal, por lo que las gaviotas pueden tener un papel en la dispersión de malas hierbas importante. Otras semillas son depositadas fuera del arrozal, con distancias de hasta 150 km de distancia. Tener en cuenta a las aves acuáticas en las distancias de dispersión de plantas tiene implicaciones importantes para la conectividad de especies entre ambientes adecuados, así como en la dispersión de malas hierbas a otros cultivos agrícolas.

En el **Capítulo 4**, se cuantifica la importación externa de nutrientes en la Laguna de Fuente de Piedra (Málaga, Andalucía) por parte de la gaviota sombría. Se combinó la base de datos GPS con análisis de nutrientes en excrementos y en egagrópilas para estimar la cantidad de nitrógeno y fósforo total que entra en la laguna. Los datos GPS se usaron para calcular el tiempo que permanecen las gaviotas en la laguna a lo largo de varios años, pero también para corregir los censos de las gaviotas que se marchan pronto y no están incluidas en los censos. Además, se determinaron cuatro vertederos como principales destinos de alimentación en la zona, y por lo tanto, serían el origen de esos nutrientes.

Por último, en el **Capítulo 5** se utilizó el análisis de excrementos para estudiar el papel de las gaviotas como bioindicadores de la contaminación ambiental por metales pesados y otros metaloides. Hay variaciones importantes en las concentraciones de metales en excrementos recogidos en distintos hábitats y zonas, lo que estaría relacionado con la exposición a contaminantes por cada zona. Además se adaptó el modelo de nutrientes del **Capítulo 4** para cuantificar

la entrada de metales pesados en la Laguna de Fuente de Piedra.

Summary

Ecosystems services provided by waterbirds are diverse, and processes such as seed dispersal and biovectoting are among the most important. Due to global change, those services may become “disservices” if the seeds dispersed by waterbirds are inconvenient (e.g. weeds), or the biovectoring of nutrients and contaminants to wetlands that are already vulnerable to global change. Previous studies have shown the role of waterbirds on ecosystem services, but the occurrence of new GPS tracking technologies allow to make more realistic quantification of such services by detailed movements.

In this PhD thesis, I focus in the role of the lesser black-backed gull *Larus fuscus* on seed dispersal and biovectoting at Andalusian scale. The availability of wintering detailed GPS movements, along with the species capacity to exploit several habitats, make the lesser black-backed gull a good model species.

In **Chapter 1**, the combination of GPS movements with network analyses allowed the creation of a connectivity network between the most exploited habitats by the lesser black-backed gull. In this way, I could determine where and in which frequency the different connections between most important habitats were occurring. Therefore, those connections were involved in the seed dispersal and biovectoring processes. Landfills and ricefields are the most important habitats to maintain the connectivity generated by gulls, and the connections in which an aquatic systems act as a final source are especially relevant. Most of the conections occur within a 60 km range, so different functional units within Andalusia may be created. Within such distance, biovectoring and dispersal processes are more likely to occur.

Chapter 2 is focused solely in Doñana ricefields and it compares the dispersal potential of two generalist species that feed within the ricefields in the same way: lesser black-backed gull and white stork. Faeces and pellet analyses

Summary

and germination experiments under laboratory conditions showed that the community of plants dispersed is extense, covering a range of 21 taxa, including weeds. However, there was no great variation in the community of plants dispersed by the two bird species, despite the differences in body mass.

After determining the main weed species dispersed by gulls, the objective of **Chapter 3** is to combine GPS gull movements with seed retention times (obtained theoretically and experimentally with captive gulls) to generate spatial seed dispersal models taking Doñana ricefields as a starting point. Due to the vast ricefield area, high percent of the seeds were deposited within the ricefields, so gulls may have an important dispersal role of weed population homogeneization. Other seeds were deposited outside of the ricefield area, with dispersal distances up to 150 km. Taking into account waterbirds for plant dispersal distances has important implications in species connectivity between suitable habitats as well as weed dispersal towards other agricultural environments.

In **Chapter 4**, the objective is to quantify the external nutrient loading to Fuente de Piedra Lake (Málaga, Andalusia) by the lesser black-backed gull. Through the combination of GPS data with nutrient analyses of total nitrogen and phosphorus in gull faeces and pellets, the quantity of nutrients loaded to the lake were estimated. GPS data were used to calculate the time that gulls spent in the Lake during several years, but also to correct gull countings for such gulls that departed earlier and are not included in census data. Furthermore, four landfills in the surroundings were the main foraging sites for gulls in the region, and thus, the main source of such external nutrients.

Finally, **Chapter 5** applied faecal analyses to determine the role of gulls as bioindicators of environmental exposure to heavy metals. There were important variations in heavy metals concentrations in faeces along sites, which would be related to the pollution exposure of the area. Furthermore, the previous nutrient model from **Chapter 4** was adapted to quantify the external input of heavy metals into Fuente de Piedra Lake. Long term heavy metal deposition may be of importance to the lake dynamics.

General introduction

Ecosystem services provided by birds have been related mainly to terrestrial ecosystems, while the roles of waterbirds within aquatic ecosystems are normally overlooked (Green & Elmberg, 2014). Moreover, terrestrial and aquatic ecosystems have been studied separately and only few studies have taken into account the interface between terrestrial and aquatic environments (González-Bergonzoni et al., 2017; Soininen et al., 2015). Waterbirds are able to move between aquatic and terrestrial ecosystems and thus it is important to consider the services they provide to ecosystems. Among the services provided by waterbirds (cultural, provisioning, supporting and regulating; Green & Elmberg, 2014), the so-called “supporting services”, such as propagule dispersal or nutrient cycling, are vital to ecological communities and ecosystems.

When aggregating for roosting or breeding, waterbirds can import external nutrients and cause shifts in the trophic status of wetlands. Waterbirds typically feed in both aquatic and terrestrial systems and are frequently involved in inter-habitat subsidies of nutrients. In arctic and other oligotrophic natural landscapes, nutrients are often a limiting factor and the nutrient load provided by waterbirds can have positive effects on diversity and productivity of the ecosystems (Van Geest et al., 2007; Plazas-Jimenez & Cianciaruso, 2020). However, eutrophication in wetlands immersed within human-modified landscapes (due to modern agriculture or urban activities) is frequent nowadays. Under a global change context, those activities combined with nutrient subsidies from waterbirds can lead to an excess of nutrients that switch from a clear-water to a turbid state (Moss & Leah, 1982). In such cases, services provided by waterbirds can turn into “ecological disservices” rather than a service (Green & Elmberg, 2014; Dunn, 2010; Buij et al., 2017).

Seed dispersal has been considered the main service provided by birds in terrestrial ecosystems. Literature regarding seed dispersal have been mainly focused in fleshy fruits and less attention was paid to the role of waterbirds as propagule dispersers (Green & Elmberg, 2014). Waterbirds are known to be vectors of co-dispersal of a whole range of plants and aquatic invertebrates and

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they can play an important role in enhancing connectivity between populations and communities (Green & Figuerola, 2005). However, the creation of human-modified habitats may favour the dispersal and spread of other species, such as alien species or agricultural weeds. Moreover, land transformation may change the spatial distribution of suitable patches for waterbirds, and hence, their movements and co-dispersal potential. Identifying where and which taxa can be dispersed by waterbirds is important to identify what barriers limit dispersal of certain plants or favour the spread of undesired species in response to global change.

The net contribution of waterbirds to ecosystem services and disservices under global change remains unclear, but it is context dependent and will vary between ecosystems. It is vital to investigate the services provided by waterbirds in more detail to quantify them, enable correct decision making and to estimate their economic value (Green & Elmberg, 2014). To understand the services and disservices of waterbirds in human-modified landscapes (e.g. landfills and ricefields) it is necessary to develop models that combine information on internal transport of propagules and contaminants with direct vector movements.

Landfills and biovectoring

Intensive human activities have produced an increase of waste and refuse, which have led to the expansion of landfills around the world in recent decades. Before the 1980s, garbage was commonly burnt in each town and not available to birds (Tortosa et al., 2002; Wilson, 2007). Afterwards, open rubbish dumps were established, in which refuse was accumulated over an open area and left until it was covered by new waste. This opened a new “window of opportunity” for many generalist birds to forage on readily available and predictable resources (Tortosa et al., 1995; Duhem et al., 2005). Therefore, many birds (e.g. storks, gulls) have increased their population numbers because of the occurrence of such new resources (Tortosa, 2002; Ramos et al., 2009). The adaptation to exploit human-modified habitats have driven waterbirds to change their natural behaviour and adapt their movements based on exploiting inland habitats (Egunez et al., 2018; Ackerman et al., 2018).

Feeding at landfills involve potential negative environmental effects related to the transportation of heavy metals, plastics, antimicrobial resistance (AMR) and Permanent Organic Pollutants (POPs) among others (Seif et al., 2018; Achmon et al., 2018) to natural environments. Anthropogenic sites are expected to present higher concentrations of heavy metals than natural habitats (Adelekan et al., 2011; Arnold et al., 2016). Furthermore, generalist waterbirds that feed on landfills also produce guano with a high nutrient content, especially of nitrogen (N) and phosphorous (P), because of the high protein content in their diet (Hahn et al., 2007).

Birds can then act as biovectors (the transport of contaminants such as nutrients and heavy metals through a moving organism) of contaminants into aquatic systems via guano (Blais et al., 2007). Waterbirds that forage at anthropogenic environments but gather in monospecific groups for roosting are able to load external pollutants into an aquatic ecosystem (Hahn et al., 2007; Winton & River, 2019; Dessborn et al., 2016) with important effects on ecosystem functioning (Dessborn et al., 2016; Green & Elmberg, 2014). Many waterbirds perform daily foraging trips to the same landfill sites and roost in aquatic systems, with the potential to generate “hotspots” of contamination by incorporating contaminants regularly from human-influenced feeding habitats and releasing these into their wetland roost sites.

An excess of external nutrient loading because of guano deposition in aquatic ecosystems can lead to ecosystem eutrophication (**guanotrophication**), which suppose an extra environmental pressure to wetlands from anthropogenic sources (Fujita et al., 2016). For example, guanotrophication by gulls that use landfills for foraging then roost in lakes or reservoirs caused damage to ecosystem services estimated at \$100 million to improve water quality in North America (Winton & River, 2017).

The degree to which waterbirds bioaccumulate heavy metals in their tissues in relation to their excretion, will determine their ability to act as biovector for heavy metals. Closed basin wetlands are especially vulnerable to contaminant deposition from heavy metals. Heavy metals bioaccumulate in the sediment

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(which can act as a long term reservoir) or in aquatic plants and animals which can produce indirect perturbations through trophic cascades affecting the ecosystem food webs (Verma, 2020; Goodyear & McNeill, 1999).

Biovectoring then involves three stages in the contaminant transport process: (1) collection at an external foraging site, which may be an artificial site such as a landfill; (2) transport through vector movements and (3) deposition of the contaminant at a roosting site (Fig. 1). These foraging sites are often well beyond the catchment area of the wetland used for roosting, so the nutrients imported by the vectors would not reach the wetland by other means such as during flooding events.

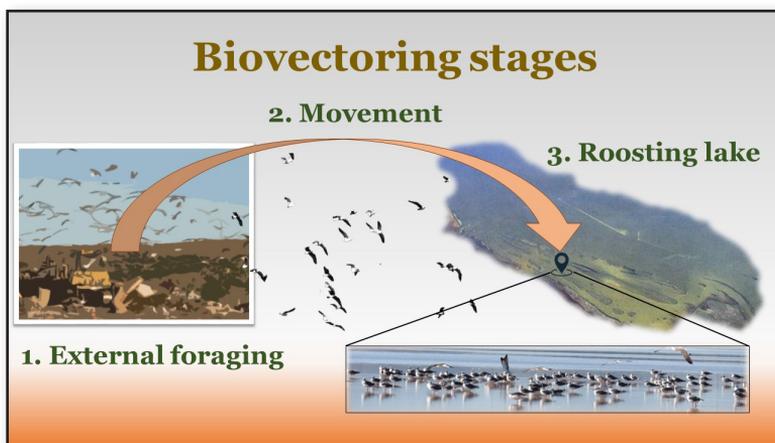


Figure 1. Example (Fuente de Piedra lake) of the three steps (intake, transport and deposition) of biovectoring from a landfill to an aquatic system.

Ricefields and seed dispersal

Wetlands are considered one of the most threatened and degraded environments because of human actions (Albert et al., 2020; Davidson et al., 2020), and nowadays such lost and degradation is still increasing (Gardner et al., 2015). The number and surface area of natural wetlands have been decreasing for decades whereas the area of human-made wetlands has increased. Worldwide, rice cultivation has increased by $0.3 \times 10^6 \text{ km}^2$ from 1970 to 2014 and reservoirs by $0.1 \times 10^6 \text{ km}^2$ from 1970 to 2010 (Davidson et al., 2018). In South Europe, 10

many natural wetlands have been transformed into ricefields, and such crops reached a surface area of 581,978 ha (Ferrero & Nguyen, 2004). Therefore, due to changes from natural to artificial habitats, ricefields have become an important alternative habitat for many generalist waterbirds that benefit from ricefields for foraging as well as breeding, wintering or roosting to lesser extent (Toral & Figuerola, 2010; Rendón et al., 2008; Fig. 2). Ricefields present a high landscape dynamism as they are first harvested, then tilled to favour organic matter decomposition and finally flooded until they are dried in order to start a new season (Fig. 2).



Figure 2. Steps in the ricefield harvesting: 1) before harvesting, 2) after harvesting, 3) tilling and 4) flooded fields post-harvest. Waterbirds forage within the rice paddies during stages 2, 3 and 4 (especially 2 and 3 in the case of gulls).

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Ricefields present a highly enriched seed bank that is represented mainly by weeds associated to rice crops (Chauhan et al., 2010) and these weeds are particularly evident along the dykes that separate rice tables (Fig. 3). Weeds associated to agricultural landscapes are characterized by the ability to remain buried in the seed bank that can emerge and germinate (Benvenuti et al., 2001). Seed dispersal is particularly important for weed population dynamics because of the frequent disturbances present in agricultural systems, as in ricefields during the harvesting period (Fig. 2). Many weeds are also exotic species that are adapted to grow and reproduce in a given agricultural environment. Adaptation for seed dispersal allows weed species to colonize new areas, establish in better suitable environments and move away from the mother plant (Benvenuti, 2007; Bourgeois et al., 2019).

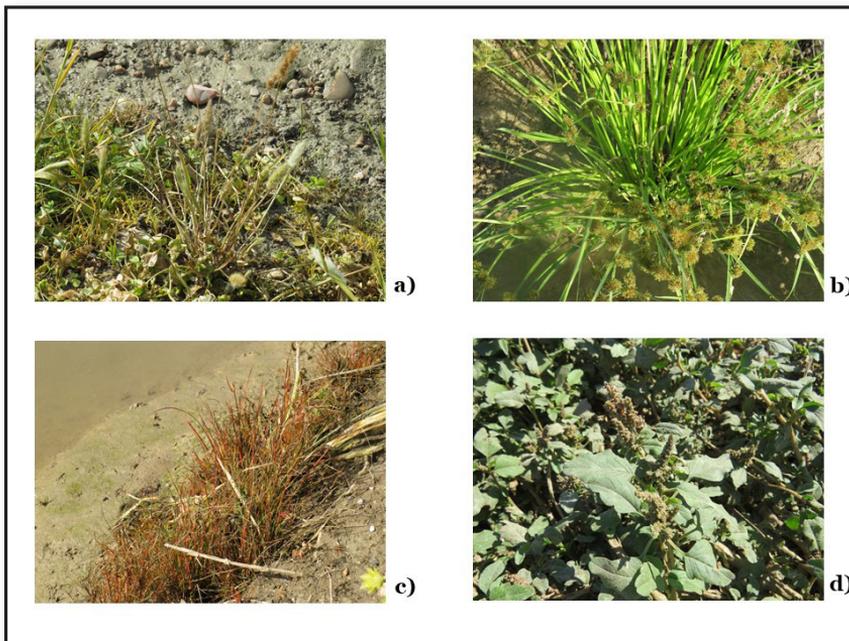


Figure 3. Examples of weeds occurring along the dykes in Iberian ricefields. a) *Polypogon monspeliensis*, b) *Cyperus difformis*, c) *Juncus bufonius* and d) *Amaranthus albus*.

Plant ecologists generally rely on dispersal syndromes, based on morphological inspection of their propagules, to make predictions about dispersal mechanisms (van der Pijl, 1982). Worldwide, weeds have been considered to be dispersed mainly by barochory (dispersal by gravity, 80%), epizoochory (dispersal by external attachment to animals, 13.6%) and anemochory (dispersal by wind, 6.1%), but also anthropochory (humans and machinery are a dispersal mode) within agricultural systems. Only 1.5% of the weeds have been considered to be dispersed through endozoochory (dispersal via gut passage, Benvenuti, 2007), because only plants with fleshy-fruits are considered to have an “endozoochory syndrome” (Costea et al., 2019) and so be dispersed by frugivorous birds (Wenny et al., 2016). Many typical weeds have no particular obvious morphological specialization such as wings, hooks or a fleshy fruit, and are therefore assigned to the barochory dispersal syndrome (Benvenuti, 2007) which assumes that the dispersal mechanism is simply for seeds to drop off the mother plant. In the recent years, many studies have highlighted the importance of waterbirds in seed dispersal services, especially through endozoochory of non-fleshy fruit plants via excreta (faeces and pellets), which has been largely overlooked in the past (Reynolds et al., 2015; Soons et al., 2016). Therefore, waterbirds feeding in agricultural landscapes (e.g. ricefields) are likely to have important implications for weed dispersal services. Ducks have been shown to provide a service by decreasing the abundance of weed seeds via foraging, but also a disservice by dispersing a fraction of those seeds (Farmer et al., 2017).

In Iberian ricefields, many species of waterbirds (e.g. gulls and storks among others) take advantage during the harvesting practices of the available resources, such as the alien red swamp crayfish *Procambarus clarkii* exposed when rice is removed (Rendón et al., 2008; Lovas-Kiss et al., 2018a). There is strong evidence for secondary dispersal of seeds carried on the outside of the crayfish by gulls in Iberian ricefields (Lovas-Kiss et al., 2018a, Fig 4). White stork *Ciconia ciconia* is another common waterbird feeding in Iberian ricefields, but an overlooked vector for seed dispersal events, although it is known to be disperse plants into its nests in other agricultural landscapes (Czarnecka & Kitowski, 2013).

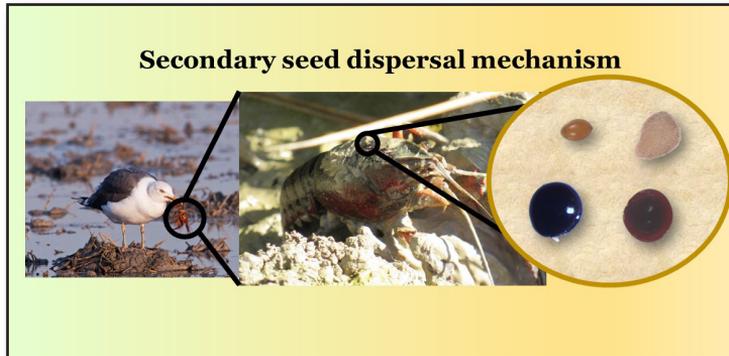


Figure 4. Example of secondary seed dispersal mechanism by gulls feeding on crayfish within the Iberian ricefields of Doñana.

From an ecological point of view, differences between waterbird species is an important factor to take into account in dispersal processes, as species will have different foraging modes, diet and habitat preferences. Furthermore, determining the degree to which a particular functional group of waterbirds is dispersing successfully (e.g. capacity to survive digestion) the same or different plant taxa is an important factor to assess the redundancy or uniqueness of roles in seed dispersal by waterbirds. From an agronomic point of view, seed dispersal of weeds and exotic species by waterbirds can be considered as a “disservice” (Green, 2016). Therefore, understanding the spatial distribution of weeds will be important to facilitate weed control at different scales (Benvenuti, 2007).

Seed dispersal process

Seed dispersal involves similar steps (intake, transport and deposition) as previously mentioned about biovectoring. Plants are ingested within an agricultural landscape and transported by the moving organism (waterbird) towards alternative suitable environments with important implications for spatial structure of populations and community dynamics of plants associated to crops (Green et al., 2002). The maximum dispersal distance a seed will be deposited depends mainly on the retention time (e.g. the time lapse between an ingestion event until excretion outside of the vector) and the movement patterns of the vector (Proctor, 1968). The longer retention times, the longer distance for potential seed dispersal. In seeds, retention times range depend partly on the

seed size (Reynolds & Cumming, 2016; Kleyheeg et al., 2018). In frugivorous birds, it seems clear that larger size vector will disperse larger seed fruits (Falcón et al., 2020), but non-fleshy fruits do not follow this rule (Tsunamoto et al., 2020). Therefore, dispersal syndromes tend to underestimate the potential dispersal distances of many plant species, as dispersal distances by waterbirds are much higher than those estimated by others dispersal syndromes such as wind and water (Bullock et al., 2017).

Spatial patterns of seed deposition by non-classical dispersal processes by waterbirds can act at different scales, from local to medium and long-distance dispersal. Seasonal behaviour will also determine the movement of the waterbird. Breeding, migration and non-breeding movements will modify the directionality and distance of a dispersal event (Viana et al., 2013, Kleyheeg et al., 2019). Dispersal destination depends completely on the ability of the vector to reach a suitable environment, which depends on the ability of a plant taxa to survive digestion germinate and establish in the new habitat and the new environmental conditions (Green et al., 2016). Generalist plants with broad distributions (e.g. weeds), will be adapted to survive in a wide variety of habitats including other agricultural landscapes and wetlands (Brochet et al., 2009). If suitable environments are frequently visited sites by the dispersal vector, seed patterns of aggregation can occur (Kleyheeg et al., 2017).

Determine the scale of transport processes by birds have been always challenging. Seed dispersal models based on theoretical parameters of movement, retention time, flight speed have attempted to determine the magnitude of a seed dispersal event (Charalambidou et al., 2003; Viana et al., 2013; Nathan et al., 2008). In this way generating dispersal kernels and seed shadows will predict the peaks of high probability of occurring a dispersal process.

1.1. Model species

Due to its own nature, the lesser black-backed gull (LBBG) *Larus fuscus* is a good model species to test and exemplify the different processes mentioned above. The white stork *Ciconia ciconia* is another model species used in **Chapter 2** (see below in thesis outline section). Both species have increased across Europe and in the South Spain in recent decades (Wetlands International, 2020; Ramo et al., 2013; Rendón et al., 2008). Both have been changing behaviour to inland habitats because are adapted to increased food availability in artificial habitats, largely from landfills and ricefields (Massemin-Challet et al., 2006; Ramo et al., 2013). More specifically, the variety of diets and habitat use of the LBBG wintering in Andalusia bring high chances to study seed dispersal, nutrient loading and heavy metal exposure of particular importance to ecosystem functioning. Furthermore, the daily colonial roosting behaviour to aquatic environments (e.g. in Fuente de Piedra lake) may make external loading processes more relevant in an environmental contamination context. Thus, it represents a good model system to study both seed dispersal from ricefields and biotransport of contaminants from landfills to aquatic roosting systems.

LBBG is a migratory waterbird that breeds in North Europe, including colonies in UK, Belgium and the Netherlands (Thaxter et al., 2015; Sotillo et al., 2014; Tyson et al., 2014). During the migration, LBBG overwinters in south Spain, Africa and some individuals remain in North Europe. In south Spain, LBBG normally arrives at the beginning of September and remains in the area until end of March when it begins the spring migration back to north Europe (Fig. 5, Klaassen et al., 2012). Generally same individuals repeat the same migration strategy over different years (Shamoun-Baranes et al., 2016).

Since 2008, a number of ongoing studies have equipped adults of LBBG with Global Positioning System (GPS) trackers, using the UvA-BiTS tracking system (Shamoun-Baranes et al., 2017; Stienen et al., 2016; Thaxter et al., 2015). The UvA-BiTS tracking system uses solar powered devices that communicate with a ground base station and allows remote data download. Trackers enable to collect different variables such as acceleration, altitude, instantaneous speed,

coordinates, date and time. All tracking data is stored in GPS positions or fixes in a centralized database (<http://www.uva-bits.nl>; see Bouten et al., 2013 for further details). The UvA-BiTS project brings up different collaborating organisations: Amsterdam University, Utrecht University and Netherlands Institute for Sea Research in the Netherlands; Ghent and Antwerpen University in Belgium and British Trust for Ornithology in UK. For this thesis I will use GPS data related with wintering behaviour at Andalusia (Fig. 6).

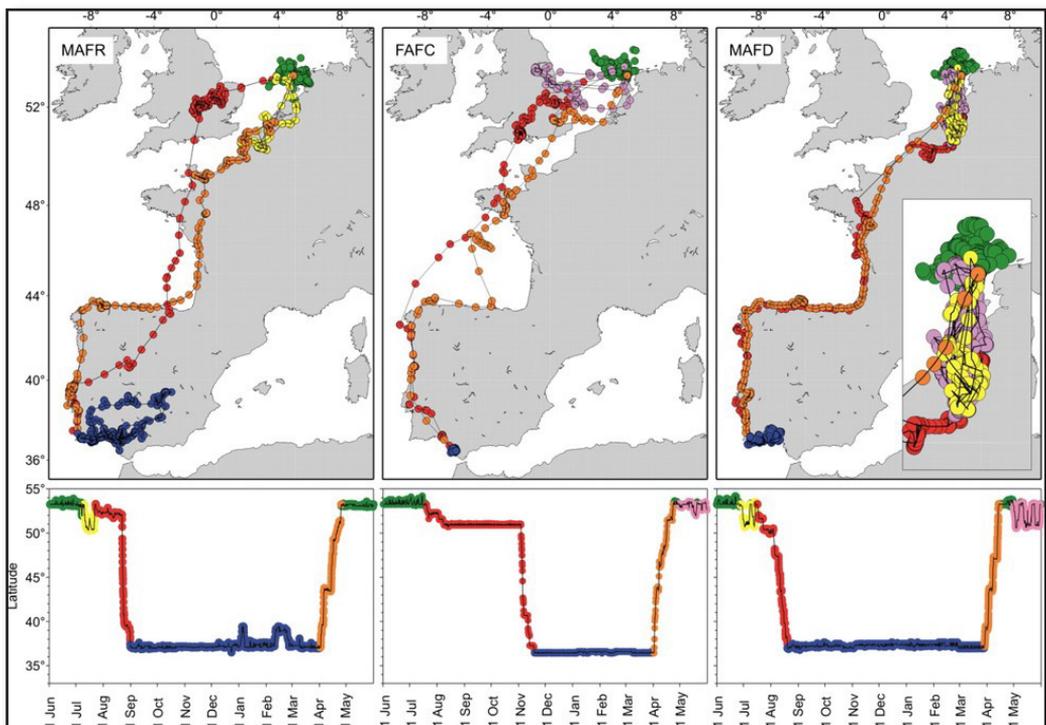


Figure 5. Migration of three individuals of *Larus fuscus* from northern Europe to southern Spain during winter between May 2007 and June 2008. Blue colour dots correspond to the wintering area, and the season they spend in southern Spain. Figure taken from Klaassen et al., (2012).

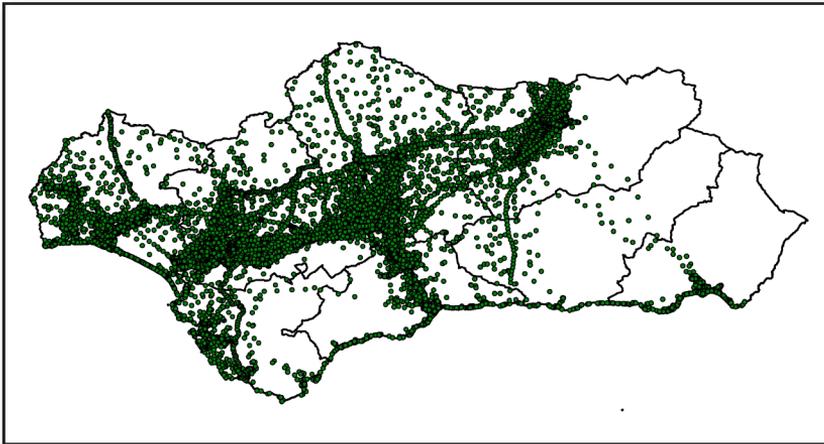


Figure 6. Visualization of all GPS data points within Andalusia from the UvA-BiTTS trackers from lesser black-backed gull *Larus fuscus*.

1.2. Outline of the thesis

The number of studies regarding the role of waterbirds as vectors of seed dispersal and nutrient loading have been growing in recent years (Soons et al., 2016; Lovas-Kiss et al., 2018a,b, 2019; Hahn et al., 2007, 2008). Nutrient loading models by waterbirds have relied on fixed metabolic parameters but did not consider the variation in habitat use by different individuals or in the source of the nutrients ingested (Hahn et al., 2007, 2008). Finally, few studies have focused on the role of waterbirds as biovectors for environmental contamination, such as heavy metal pollution. Therefore, there is still a lack of detailed information regarding the quantification and accuracy of dispersal and biovectoring processes.

The development of new technologies to track animal movements is a great tool to improve models and quantify precisely the role of waterbirds in transport effects across multiple spatiotemporal scales. The lesser black-backed gull (LBBG) *Larus fuscus* is a good example to model biovectoring by waterbirds thanks to the availability of detailed GPS data from tracking devices for this species (Bouten et al., 2013; Baert et al., 2018; Klaassen et al., 2012; Shamoun-

Baranes et al., 2017; Thaxter et al., 2019). Such GPS data allows the identification of specific feeding sites that can act as sources of seed ingestion or contaminants intake. Furthermore, GPS data provide information related to movement directionality, speed, distance, date and time from the “source” of biovectoring towards the final destination that act as a “sink” both for seed dispersal and abiotic biovectoring.

I will frame this PhD thesis in the region of Andalusia, in southern Spain, where the LBBG is abundant during the non-breeding season (Shamoun-Baranes et al., 2017). Both dispersal and biovectoring events described above are addressed, to represent processes carried out by waterbirds, taking the LBBG as an example covering different spatiotemporal scales in Andalusia. The study of biovectoring and dispersal by gulls in Andalusia offers the opportunity to expand the current knowledge of these processes within a heterogeneous and anthropogenic landscape, with detailed movement patterns. The availability of GPS information regarding this model species allows us to combine this information with different approaches in order to develop more realistic models.

Chapter descriptions

In **Chapter 1**, I combine GPS data from LBBG with a network analyses approach to develop an inter-habitat connectivity network and understand the role of gulls as biovectors and dispersers within the Andalusian landscape (Fig. 7). I focused on the terrestrial-aquatic interface to understand how, when and to what extent aquatic ecosystems can be impacted by terrestrial and anthropogenic foraging behaviours (e.g. landfills, ports). First, I determined the main habitat types involved in the connectivity of gulls in Andalusia and identified specific Andalusian locations that act as crucial nodes within the network. Through real GPS gull trajectories, I could determine the strength, temporality and the directionality of the connections between the locations in the network, which make biovectoring and dispersal processes by gulls more likely to happen. Some locations act as “sources” of intake for biovectoring processes and others as “sinks” of deposition. The strength of connections between locations will be a clear example of a quantification of the movement stage in the biovectoring process.

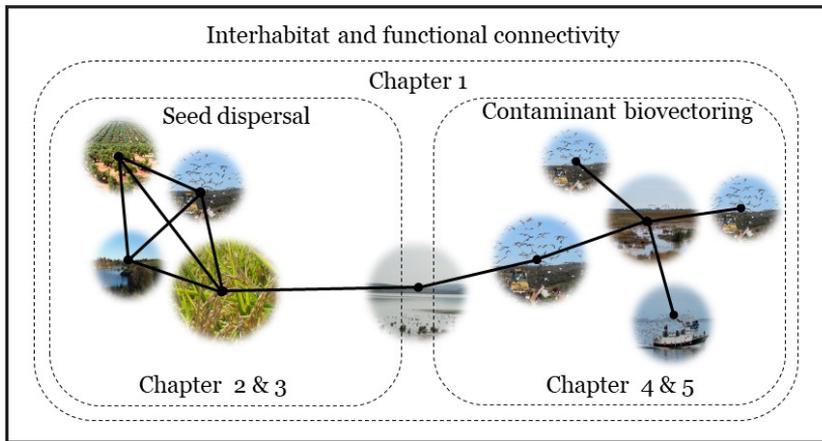


Figure 7. Conceptual framework of biovectoring and seed dispersal by gulls and thesis outline indicating the related topic of each chapter.

In **Chapter 2**, after the identification of the ricefields as one of the key nodes in the connectivity of gulls for Andalusia in the previous chapter, I carried out a fieldwork and laboratory approach in order to determine the breadth of plant species whose diaspores are egested and deposited by gulls and white stork excreta (Fig. 7). I tested the potential for dispersal within the landscape by carrying out germination experiments. In this way I quantified the frequency and quality of a seed dispersal event. Furthermore, I compared the dispersal potential with both species as they are common generalist waterbirds within the ricefields to check if vectors with different body size but feeding in a similar manner within a relatively homogenous environment are able to disperse a different range of plant species. Finally, I carried out vegetation transects to quantify the range of plant taxa dispersed by those bird species in relation to what is available in the landscape.

Linked to the previous chapter, **Chapter 3** aims to develop seed dispersal models based on the ricefields as intake locations, using GPS gull data and seed retention curves to calculate the seed dispersal shadows (Fig. 6). Due to the great extension of the ricefield area studied, seed dispersal models based on gull trajectories are framed within different spatial scales: movements that remain within the

ricefields (and thus seeds may remain within the same homogenous landscape) and beyond the ricefields towards other different habitats. The mechanism of seed dispersal of this landscape has also temporal restrictions, as it is related to the management practices of the rice harvesting. Harvesting practices make available a high peak of food resources that are no longer available once the harvesting is over (Fig. 2). This will determine the behaviour and movement of the gulls within and outside of the ricefields and thus, the potential for seed dispersal (dominated mainly by weeds associated with crops). In this case, the ricefields can act both as sources and sink sites. When gulls depart the ricefields, the potential distance and the suitability of the habitat where these seeds are deposited become very important to quantify the potential impact of seed dispersal by gulls.

In **Chapter 4**, I combined GPS data information with nutrient analyses (from faeces and pellets) in the laboratory to quantify the external loading of nutrient by gulls into an important inland lake in Andalusia (Fig. 7). Fuente de Piedra Lake has a closed basin and is hypersaline, and is the most important natural lake in the region, and many gulls use this lake for roosting during winter. GPS data information allows us to estimate the amount of time gulls spend at the lake and thus, the quantity of guano deposited at the lake that contributes to nutrient deposition and potential eutrophication (Fig. 1). I also used GPS information to correct the censuses of gulls at the lake, because many individuals departed the lake early morning to the foraging sites before counting. GPS data also helped to determine the most important foraging sites where gulls were ingesting such nutrients. Identifying the source of the nutrients finally deposited into the lake, and which have such a strong influence on ecosystem functioning, is an important step towards identifying potential future management actions.

In **Chapter 5** I will evaluate the role of gulls as biovectors of heavy metal pollution in the environment (Fig. 7). The degree of heavy metal exposure was determined through faecal analyses in different habitats within Andalusia. Gulls can act as biovectors of heavy metal deposition when roosting in groups in aquatic ecosystems (Fig. 1). Therefore, I will adapt the nutrient quantification model from **Chapter 4** to quantify the heavy metal deposition in Fuente de

Piedra lake as an example of biovectoring by gulls. Finally, as a way to summarize the importance of faecal analyses in heavy metal analyses, I will review all the studies carried out in birds so far, and put my own study in their context.

Finally, I will integrate all previous chapters that summarize, discuss and put in context the role of gulls in biovectoring, taking as a model *Larus fuscus* and their movement patterns within the Andalusia study area. Furthermore, I will discuss potential management perspectives to balance the role of gulls in aquatic ecosystems under a global change scenario.

1.3. Study area

The study was carried out in different wetlands and artificial habitats with different degree of anthropization in the Spanish autonomous region of Andalusia (South Spain; Fig. 7). Some specific sites allowed me to study the biovector and seed disperser role of LBBG, and the most relevant are:

- The Doñana ricefields (Fig. 7) in the Guadalquivir (Seville, 37,000 ha) has doubled in extent since the 1960s, and is the largest area devoted to rice production in Spain, accounting for up to 42% of total national harvest. This area is located in one of the most important wetland complexes (Doñana) for migratory waterbirds in the Western Palearctic, and is particularly important for LBBG and white stork, hosting more than 10,000 and 1,000 daily individuals respectively in early winter (Ramo et al., 2013; Rendón et al., 2008). This area is especially relevant for **Chapters 1, 2 and 3**.
- Fuente de Piedra lake (Málaga, 1,350 ha) is one of the most important natural shallow lakes in Spain, and is protected at regional (Natural Reserve), European (Special Protection Area) and international (Ramsar site) levels (Fig. 7). It hosts one of the two largest flamingo nesting colonies in the western Mediterranean (Bechet et al., 2012) and is a roosting area for over 20,000 LBBG in winter (Censos Junta de Andalucía). I focus on Fuente de Piedra in **Chapters 1, 4 and 5**.

- The Cetina saltpan complex (1,100 ha), was created in 2014 and is one of the biggest saltpan complexes in Spain, and one of the most important in the area for salt production, and LBBG use it mainly as a roosting site (Fig. 7). This site was used in **Chapter 6**.
- Several landfill sites were used during this thesis (e.g. one in Córdoba or one near the Doñana ricefields among others, Fig. 7). These landfills are used by large numbers of LBBG for feeding, and are key sites for contaminant exposure to gulls when feeding, and for potential biovectoring processes. Landfills are particularly important in **Chapters 1, 4 and 5**.

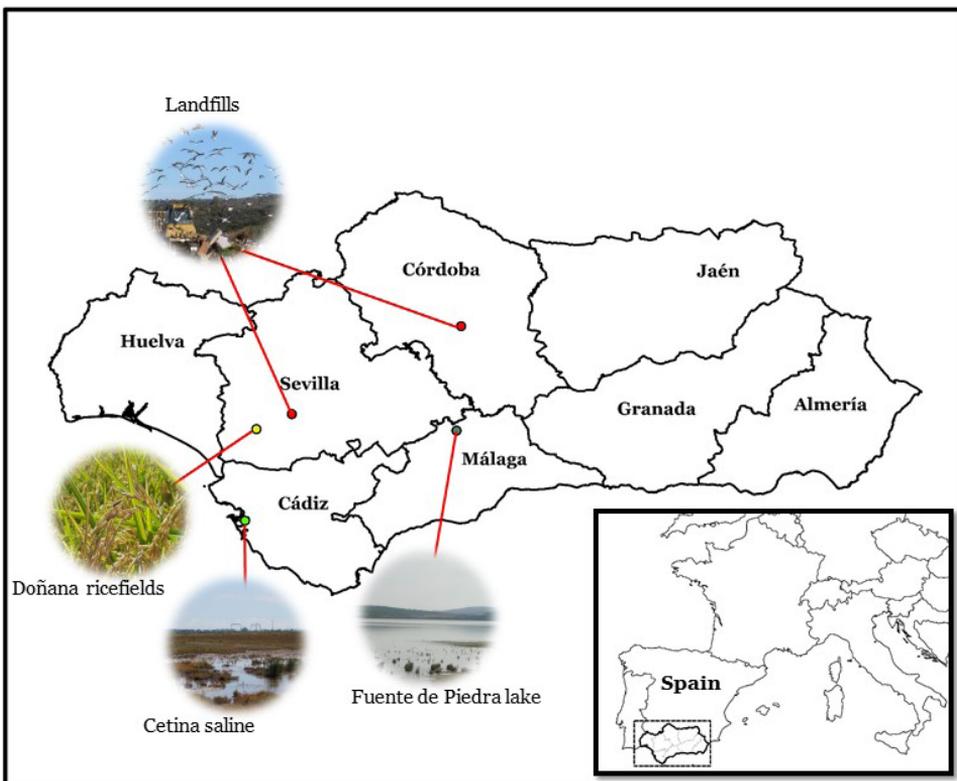
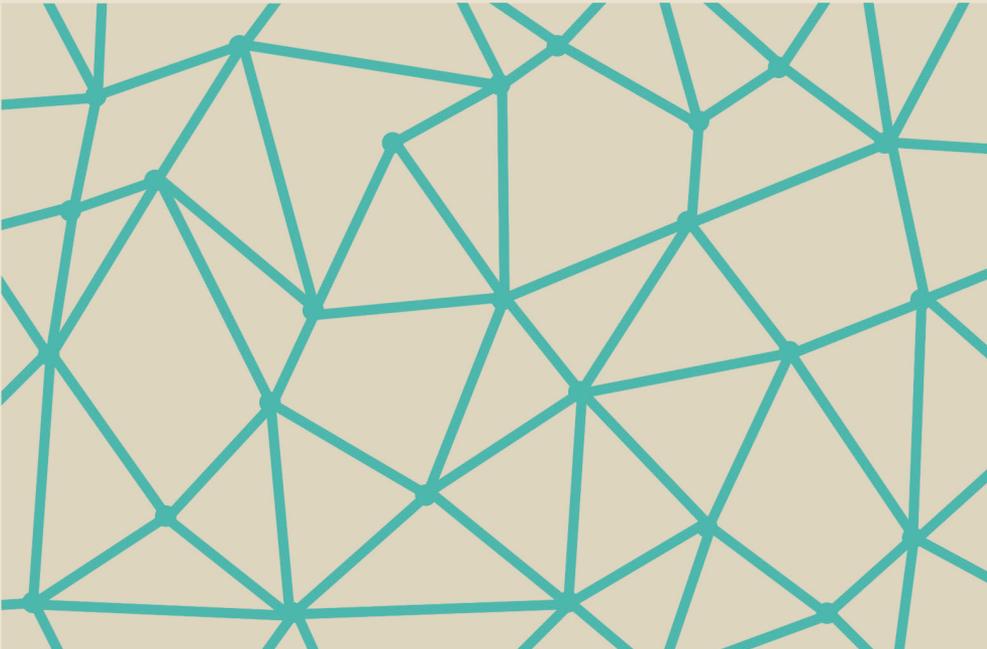


Figure 8. Spatial location of the main sites used within this PhD thesis in the regional province of Andalusia.

Chapter 1

Functional connectivity network between terrestrial and aquatic habitats by a generalist waterbird, and implications for biovectoring



Martín-Vélez V., Mohring B., van Leeuwen C. H. A., Shamoun-Baranes J., Thaxter C. B., Baert J. M., Camphysen C. J. & Green A. J. (2020). Functional connectivity network between terrestrial and aquatic habitats by a generalist waterbird, and implications for biovectoring. *Science of the Total Environment*, 705-135 886.

Abstract

Birds are vectors of dispersal of propagules of plants and other organisms including pathogens, as well as nutrients and contaminants. Thus, through their movements they create functional connectivity between habitat patches. Most studies on connectivity provided by animals to date have focused on movements within similar habitat types. However, some waterbirds regularly switch between terrestrial, coastal and freshwater habitats throughout their daily routines.

Lesser black-backed gulls that overwinter in Andalusia use different habitat types for roosting and foraging. In order to reveal their potential role in biovectoring among habitats, we created an inter-habitat connectivity network based on GPS tracking data. We applied connectivity measures by considering frequently visited sites as nodes, and flights as links, to determine the strength of connections in the network between habitats, and identify functional units where connections are more likely to happen. We acquired data for 42 tagged individuals (from five breeding colonies), and identified 5,676 direct flights that connected 37 nodes. These 37 sites were classified into seven habitat types: reservoirs, natural lakes, ports, coastal marshes, fish ponds, rubbish dumps and ricefields.

The Doñana ricefields acted as the central node in the network based on centrality measures. Furthermore, during the first half of winter when rice was harvested, ricefields were the most important habitat type in terms of total time spent. Overall, 90% of all direct flights between nodes were between rubbish dumps (for foraging) and roosts in other habitats, thereby connecting terrestrial and various wetland habitats. The strength of connections decreased between nodes as the distance between them increased, and was concentrated within ten independent spatial and functional units, especially between December and February. The pivotal role for ricefields and rubbish dumps in the network, and their high connectivity with aquatic habitats in general, have important implications for biovectoring into their surroundings.

Introduction

Birds can efficiently exploit spatial and temporal variation in their environment to take advantage of seasonal peaks in food availability (Alerstam et al., 2003), track suitable climatic variation (Tingley et al., 2009) or reduce intra- or interspecific competition (Somveille et al., 2015). As such, birds can act as biological links among a wide range of habitat patches in ecosystems (Buelow & Sheaves, 2015), which can facilitate functional connectivity – defined here as the degree of movement or flow of organisms and their ecological functions through the landscape matrix (Taylor et al., 1993). Examples of functional connectivity are the dispersal of propagules of sessile organisms (Green & Figuerola, 2005; Lovas-Kiss et al., 2018a,b), nutrients (which can lead to guantrophication, Dessborn et al., 2016, González-Bergonzoni et al., 2017; Hahn et al., 2007, 2008), pathogens (Bauer & Hoyer, 2014) or contaminants (Blais et al., 2007). These biovector processes occur mainly through the deposition of faeces and regurgitated pellets (see **Chapter 4**). Connectivity is particularly high when different sites are used on a regular basis for different behaviours, creating a “functional unit” for the birds, e.g. when different wetlands are used by waterbirds for feeding and roosting (Guillemain et al., 2010).

Many studies on the functional contribution of birds to ecosystem connectivity have focused on terrestrial habitats, such as fragmented forests (Doerr et al., 2011; Mueller et al., 2014). Other studies have examined connectivity by waterbirds between wetlands or connectivity among different biomes (Haig et al., 1998; Merken et al., 2015; Obernuefemann et al., 2013). However, birds also frequently move between terrestrial and aquatic systems, which sometimes are separated by great distances (González-Bergonzoni et al., 2017; Soininen et al., 2015; Viana et al., 2016a). For instance, many waterbirds consume terrestrial resources during the daytime but roost in aquatic environments at night. Geese feed on agricultural lands and move to nearby freshwater ecosystems to roost, thereby loading external nutrients into aquatic systems (Dessborn et al., 2016; Unckless & Makarewicz, 2007). Gulls also feed opportunistically in a wide range of terrestrial habitats while roosting in wetland habitats (see **Chapter 4**; Winton & River, 2017) enabling functional connectivity between terrestrial

and aquatic habitats (Hessen et al., 2017). In recent years, improvements in the quality of tracking data allow connectivity studies at high spatial or temporal scales (Morris, 2012). Such studies of functional connectivity would help us to understand the effects of waterbird movements on wetland functioning (Green & Elmberg, 2014), and be useful tools for the development of management plans (Amezaga et al., 2002; Si et al., 2018). Nevertheless, there is still little information regarding the role of waterbirds as functional connectors among different habitats. In this study, we aim to create an inter-habitat connectivity network based on GPS tracking data.

Gulls (Laridae) are a group of waterbirds known for movements between terrestrial, freshwater and marine habitats. The lesser black-backed gull (LBBG) *Larus fuscus* has a European wintering population of over half a million birds (Hagemeijer & Blair, 1997; Wetlands International, 2019) and has become an abundant wintering waterbird on inland water bodies. This species is a generalist omnivore that uses a range of terrestrial, freshwater and marine habitats (Camphuysen et al., 2015). Global Positioning System satellite tracking has shown that individuals from breeding populations in Belgium, the United Kingdom and the Netherlands spend part of the non-breeding season in Andalusia in southern Spain (Baert et al., 2018; Klaassen et al., 2012; Shamoun-Baranes et al., 2017; Thaxter et al., 2019). Given their great movement capacity, broad niche and habit of foraging and roosting in different locations, the LBBG is an excellent species for studying functional connectivity between different habitat patches.

In Andalusia, the LBBG exploits food in various habitats. Ricefields in the Doñana wetland complex are important for many waterbird species, including gulls, and provide abundant food during the harvest period, such as the alien red swamp crayfish *Procambarus clarkii* exposed during harvesting and tilling (Lovas-Kiss et al., 2018a; Rendón et al., 2008; Toral & Figuerola, 2010). There are also numerous uncovered rubbish dumps (landfills) in open areas (Navarro et al., 2016). Rubbish dumps provide accessible food resources, which contain nutrients, contaminants and pathogens that can potentially be introduced by gulls into reservoirs, lakes and other environments used for roosting (Arnold et al., 2016; Duhem et al., 2005; Winton & River, 2017). Coastal ports are also a feeding

habitat, since they provide access to marine discards (Oro, 1996) and individuals that use these resources can potentially connect coastal and inland habitats. However, temporal changes in food availability in different habitats during the winter period may lead to movements of gulls around the network of sites across the Andalusian landscape. Changes in food availability and accessibility likely determine the decision to spend time in certain foraging habitats, or to move to new habitats with higher amount of food resources, influencing the functional connectivity. Because flight is relatively costly and birds should balance their energy expenditure with their energetic intake (Ydenberg, 1994), we can also expect the distance between foraging or roosting sites to be an important determinant of the level of functional connectivity within the network of sites.

The main aim of this study is to determine the extent to which gulls connect different habitat types, in order to understand their potential role as biovectors across terrestrial and aquatic habitats. We used a connectivity network approach based on direct flights derived from GPS data to explore the functional connectivity provided by LBBGs within Andalusia. Our specific objectives were: (1) identification of the main habitat types (and nodes) that make up the regional network, and the relative role of each habitat in maintaining functional connectivity, by quantifying how much time individually-tracked gulls spent in different habitat types, the strength of the different connections and centrality measures. (2) study the strength of connectivity between terrestrial and wetland habitats, and between coastal and inland habitats. (3) determine if distance has a negative effect on the degree of connectivity between sites, and identify functional units at a local scale through connectivity analyses. (4) investigate the change in connectivity provided by gulls over the course of the winter season.

Material and methods

Study region

We studied flights of lesser black-backed gull (LBBG) in the Spanish autonomous region of Andalusia. Andalusia holds 56% of the total surface area of natural wetlands in Spain (Consejería de Medio Ambiente, 2005) – including the

Doñana wetland complex – and contains the majority of waterbirds wintering in Spain (del Moral, 2003). The Doñana wetland complex is the most important site in Spain for wintering waterbirds, and holds extensive natural marshes as well as ricefields, fish ponds and salt ponds (Green et al., 2018; Rendón et al., 2008). The area of ricefields has doubled since the 1960s, and is particularly important for LBBG (Ramo et al., 2013; Rendón et al., 2008). Elsewhere in Andalusia there are over 30 natural, closed-basin shallow lakes (known locally as “lagoons”, Rodríguez-Rodríguez, 2007), and numerous large and small reservoirs constructed to supply agriculture and urban areas (Lehner et al., 2011).

Gull tracking data

The LBBG is a well-studied bird species in Western Europe. A number of ongoing studies have equipped adult LBBG with Global Positioning System (GPS) trackers between 2008 and 2018, using the UvA-BiTS tracking system (Shamoun-Baranes et al., 2017; Stienen et al., 2016; Thaxter et al., 2015). Details of the GPS tracking system can be found in Bouten et al., (2013) and examples of fieldwork and attachment methods can be found in Baert et al., (2018), Thaxter et al., (2019) and Van Donk et al., (2019). All tracking data is stored in a centralized database (<http://www.uva-bits.nl>; Bouten et al., 2013). Data is stored in GPS positions or fixes. Position accuracy with a stationary signal every 10 min was on average 30 m (Bouten et al., 2013).

From UvA-BiTS database, we first selected all GPS positions between latitudes 36.0 - 40.5° N and longitudes of 9.0 - 0.0° W that belong to the Andalusia region. We selected gull positions between October and March (the wintering period), which ensured that at least 20 different tagged individuals were present in any given month between October 2010 and March 2017 (i.e. seven winters). For our analysis, we used the following parameters recorded by the GPS trackers: date, time, latitude, longitude and instantaneous speed. We calculated additional variables from consecutive GPS positions: Haversine distance (spherical distance between geographic coordinates of GPS fixes), time difference between fixes, and trajectory speed (km/h as distance/time).

We filtered the dataset for quality by deleting any fix with ground speeds (either instantaneous or trajectory) exceeding 80 km/h, as this covers most recorded flight speeds for this species (Shamoun-Baranes et al., 2017). We filtered the database for gaps of more than 60 min between fixes, assuming these to be caused by e.g. battery depletion. We also deleted trajectories from gulls that were in transit on their migration towards Africa (see Klaassen et al., 2012; Thaxter et al., 2019). The resulting dataset included trajectories from 74 individuals belonging to five breeding colonies in North Europe (Walney, Skokholm, Zeebrugge, Texel and Orford Ness). Some individuals were present in Andalusia during several winters, making a total of 114 bird-winters (referred to as “bird-years” from here on).

Site selection

To create a connectivity network for the region of Andalusia, we first identified the 13 most important sites for LBBG according to wintering waterbird count data (data provided by Junta de Andalucía), these being the sites with a mean January (between 2010 and 2017) count of over 1,000 birds. In addition, we used hotspots of GPS data to identify 24 other sites important for LBBGs, 17 of which were not covered by the waterbird censuses. Those hotspots showed any GPS activity and were considered as wetlands, rubbish dumps or ports (roost or foraging sites) according to CORINE Land Cover 2012 (Coordination of Information on the Environment, CLC; <https://land.copernicus.eu/>). Combining census information and hotspots from tracking data, we therefore identified the 37 most important sites within the study region, which we classified into seven main habitats to facilitate analysis: rubbish dumps (12), reservoirs (11), coastal marshes (7), lagoons/shallow lakes (3, two of which were complexes of several small lakes), ports (2), ricefields (1) and fish ponds (1) (Fig. 1, Table 1). We delimited the sites based on the polygons associated with CLC 2012 habitat types, and applied a 200-meter buffer around the perimeter to account for gulls that may be resting around the site before departing. Spatial data processing was carried out using ArcMap 10.4. The 37 selected sites held 71.6% (410,623 out of 573,096 fixes) of all the GPS fixes available for the whole Andalusia region.

Chapter 1

The only fish pond site was Veta la Palma in Doñana Natural Park (Walton et al., 2015), the ricefields were also in the Doñana wetland complex (Rendón et al., 2008). The most important natural shallow lake was Fuente de Piedra (the largest natural lake in Andalusia; Batanero et al., 2017).

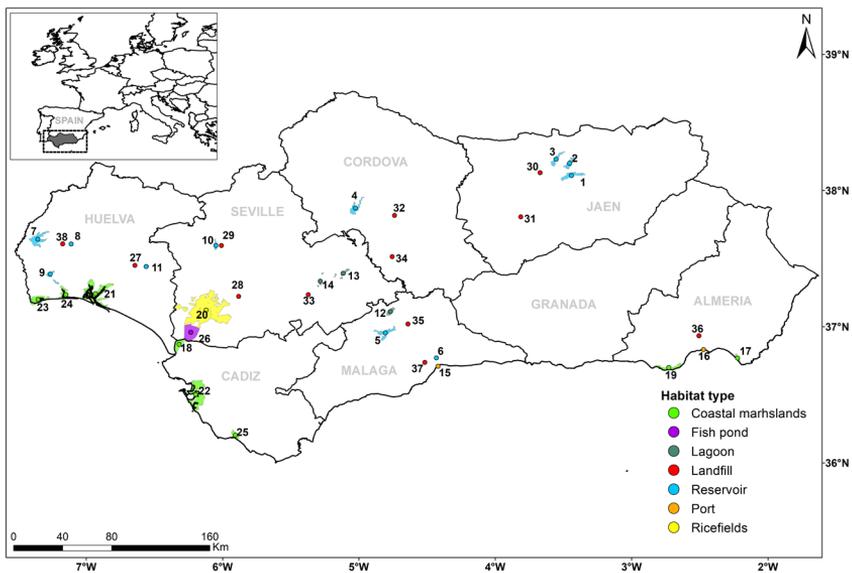


Figure 1. Location of the 37 sites within Andalusia region in South Spain, classified into seven main habitat types.

Table 1. Details of the 37 LBBG sites selected for Andalusia, listing the habitat type, identity code (ID), location, surface area (km²), relative time spent (%) in the site during the whole winter, mean January counts (when available, data for 2010-2017), and Betweenness Centrality (BC) values (number of shortest paths between other nodes that pass through a particular node). The top five values for each of area, relative time spent, mean counts and BC are highlighted in bold.

	Habitat Type	ID	Location	Area (km ²)	% time spent	Mean counts	BC
1	Reservoir	GiR	Giribaile	37.01	10.2	972	158
2	Reservoir	NR	Guadalén	21.62	2.26	117	0
3	Reservoir	FR	Fernandina	22.72	0.33	1,284	24
4	Reservoir	BR	Breña	34.6	1.64	5,286	110
5	Reservoir	HR	Guadalhorce	39.65	0.31	656	0
6	Reservoir	LR	Limonero	2.69	0.11	-	0
7	Reservoir	AR	Andévalo	59.71	0.93	-	88
8	Reservoir	GrR	Grande	1.39	0.02	-	5
9	Reservoir	PR	Piedras	12.93	0.04	79	138
10	Reservoir	GeR	El Gergal	8.02	0.39	-	0
11	Reservoir	CBR	Corumbel Bajo	5.16	0.14	-	0
12	Lagoon	FPL	Fuente de Piedra	18.66	12.08	18,690	125
13	Lagoon	LEL	Lantejuela East	10.01	1.06	4,172	4
14	Lagoon	LWL	Lantejuela West	3.64	1.36	1,222	0
15	Port	MP	Malaga	3.51	0.74	-	69
16	Port	AP	Almería	3.73	2.31	-	0
17	Ricefield	DR	Doñana	493.28	43	10,800	454
18	Coastal marsh	CGM	Cabo de Gata	9.44	0.13	302	4
19	Coastal marsh	PEM	Punta Entina Sabinar	34.12	0.06	1,011	0
20	Coastal marsh	HM	Huelva	177.46	4.8	5,187	194
21	Coastal marsh	CM	Bahia de Cadiz	181.86	0.01	5,882	31
22	Coastal marsh	ICM	Isla Cristina	62.36	0.08	3,366	3
23	Coastal marsh	RM	El Rompido	47.44	0.09	2,082	111
24	Coastal marsh	BM	Barbate	25.3	0.02	346	0
25	Fish pond	VLP	Veta la Palma	104.49	0.34	284	0
26	Dump	RTD	Rio Tinto	1.15	1.85	-	282
27	Dump	AGD	Alcalá de Guadaira	2.69	5.26	-	99
28	Dump	ARD	Alcalá del Río	1.47	1.01	-	52
29	Dump	LD	Linares	1.31	5.61	-	0
30	Dump	JD	Jaen	1.32	0.08	-	188
31	Dump	BD	Breña	1.69	0.23	3,536	68
32	Dump	MarD	Marchena	1.22	0.43	-	2
33	Dump	MoD	Montalban	1.26	1.22	3,450	267
34	Dump	AnD	Antequera	1.46	0.71	-	34
35	Dump	ALD	Almería	0.94	0.34	-	4
36	Dump	MalD	Malaga	2.65	0.34	-	43
37	Dump	TD	Tharsis	1.08	0.44	-	33
Total				1,439	100%	68,724	

Flight selection

We filtered the dataset to identify “direct flights” (i.e. without stopping) from one site to another. Although flight speeds are variable (Shamoun-Baranes et al., 2016), we considered direct flights to be represented by instantaneous or trajectory speed ≥ 10 km/h (after examining histogram of speeds for cut-offs). We defined a flight as the trajectory between two sites in which instantaneous or trajectory speed was ≥ 10 km/h, beginning and ending with a speed in each site < 10 km/h. This process excluded cases where a bird simply flew over a site. For the identification of direct flights, we also discarded trajectories with more than one fix with a ground speed lower than 10 km/hr (either instantaneous speed or trajectory speed), as this implied a pause in between sites (Shamoun-Baranes et al., 2011; Klaassen et al., 2012). After this screening process we removed flights of more than one day (24h), because retention times of content in the digestive system suggest such long flights would contribute little to biovectoring (Nogales et al., 2002). After this selection process, we had identified 5,676 direct flights between the selected sites, performed by 42 tagged gull individuals (and 84 different bird-years).

Habitat use

We quantified habitat use as the percentage of time spent by tagged gulls in each habitat type, and site for each of six months (October-March), including the seven study winters, in order to identify seasonal patterns (objectives 1 and 4). We performed all data filtering and calculations in R (v.3.4.4. R Core Team, 2018).

Connectivity network

We considered each of the 37 sites within Andalusia as an independent “node”, and considered direct flights between these nodes as “links” in the connectivity network. We calculated “betweenness” (objective 1) measures to identify central nodes in the network (Bastille-Rousseau et al., 2018) by making the links binary (connected/not connected). “Betweenness” is a centrality measure that

quantifies the number of times a node acts as a bridge along the shortest path between two other nodes, and was calculated with the R package *igraph* (Csardi & Nepusz, 2006).

We then weighted the strength of each link between nodes by calculating the total number of direct flights (objective 1 and 2). We considered the links between nodes as directed or asymmetrical (i.e. for a given pair of nodes ij , the number of direct flights from node i to node j is different to the number of direct flights from node j to node i).

We calculated distances between nodes using the Haversine formula, taking polygon centroids as a reference. In order to study whether distances between nodes could predict the number of direct flights in terms of connectivity, we used a Generalized Linear Model in which “number of direct flights” was the dependent variable and “distance” between sites and “number of gulls” were explanatory variables (objective 3). We fitted the model with a quasi-Poisson error distribution to account for overdispersion of the data and to normalize model residuals through *lme4* R package (Bates et al., 2014).

We identified the main “functional units” within our network (i.e. those sets of nodes which have high connectivity within a set, and low or no connectivity between sets) by applying the `cluster_infomap` function in *igraph* (Csardi & Nepusz, 2006), based on a probability of flow of random walks to detect structures in communities (Rosvall & Bergstrom, 2008). This function finds a community structure (in this case, it creates modules that we consider to be functional units) that minimizes the expected description length of the random walk trajectory. A random walk is generated in order that makes use of the network based on the probability (weight) of traversing a particular link (in this case weighted by the number of direct flights), and repeats the random trajectory generation ten times. We created a map for this directed and weighted connectivity network, including the ten resulting functional units in ArcMap 10.4 (objectives 1, 2 and 3). We split the connectivity network into six months (from October to March) and counted the number of flights within and between each functional unit. In this way, we could identify the temporal change in relative importance of each

functional unit (objective 4).

Results

There was a total mean of 67,946 LBBG individuals counted in the 20 sites (54% of 37 sites) with midwinter census data available for Andalusia in January between 2010-2017 (Table 1). Those counts were dominated by two sites: Fuente de Piedra lake (FPL) with a mean of 18,690 gulls and Doñana ricefields (DR) with 10,800 gulls. These two sites also correspond to areas with a high relative time spent during the entire winter (12.08% and 43% respectively, Table 1). For the remaining 17 sites (10 dumps, 5 reservoirs and 2 ports) identified as GPS hotspots, no census data were available (Table 1). Sites with no census data accounted for 21.7% of the total relative time spent, including sites where a high proportion of total time was spent (e.g. 5.26% at Alcalá de Guadaíra Dump [AGD] and 5.61% at Linares Dump [LD], Table 1).

Habitat use

There was high seasonal variability in habitat use by the tagged gulls among the different months in winter (Fig. 2). Ricefields were the most important habitat during the first half of the winter, especially in October-November, when most gull activity in Andalusia was concentrated in this single, large site (Fig. 1 and Fig. 2). The time that gulls spent in ricefields decreased from December onwards, whereas time spent in other habitats such as dumps, reservoirs and lagoons increased (Fig. 2). From January onwards, dumps were more important than any other habitat type (Fig. 2). Furthermore, ricefields were unique in that gulls often remained there for more than one day before moving to a different site. This also varied seasonally, as 63% of the visits to ricefields that lasted for more than 24 hours were in October-December.

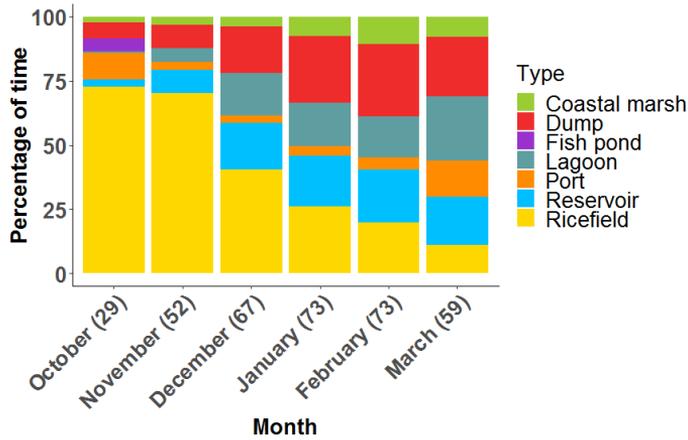


Figure 2. Percentage of time spent by tagged gulls in different habitat types for different wintering months. The number of tagged bird-years is indicated in brackets (for 2010-2017).

Connectivity analysis

Based on centrality measures of “betweenness”, ricefields (DR) were the most central node within the network, followed by three inland landfills in western (Río Tinto, RTD), central (Montalbán, MoD) and eastern (Jaén, JD) Andalusia, and the Huelva coastal marshlands (HM) (Table 1). The two landfills (AGD and ARD) near the Doñana ricefields (DR) showed the highest number of direct flights in the connectivity network (Fig. 3; Table S1). Other examples of particularly strong connections between rubbish dumps and wetlands include the Antequera dump (AnD) with Fuente de Piedra (FPL) Lake in Málaga, and Linares dump (LD) with Giribalde reservoir (GiD) in Jaén (Fig. 3, Table S1). With respect to connectivity between habitats, rubbish dumps are the habitats that hold the highest number of direct connections (90% of all direct flights) with wetlands (including reservoirs, ricefields, lagoons and marshlands), whereas only 7% of direct connections were between wetland habitats (Fig. 4, Table S2). In comparison, only 0.01% of direct connections were between exclusively terrestrial habitats (i.e. between dumps).

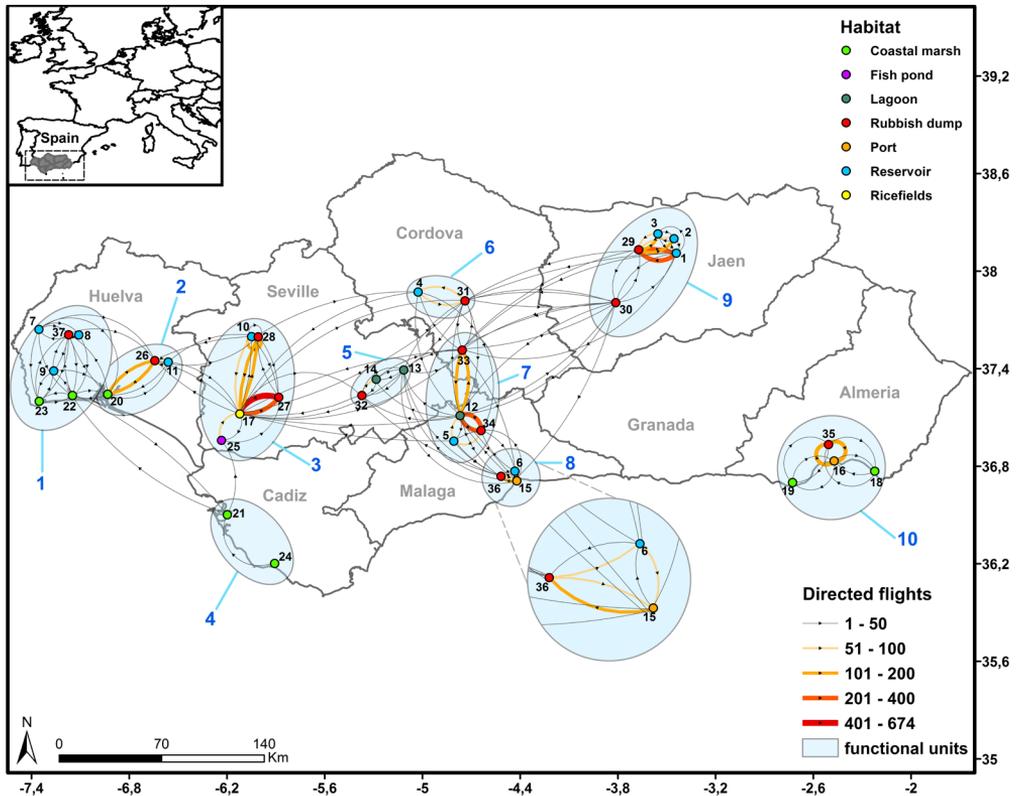


Figure 3. Total connectivity by direct flights between sites (nodes coloured according to habitat type) within Andalusia from 2010 to 2017. Line width and colour of the arrows reflects the strength of the links in terms of number of direct flights (see Table S1 for full details). Light blue ellipses represent ten functional units (numbered in blue) within the connectivity network obtained from infomap clustering.

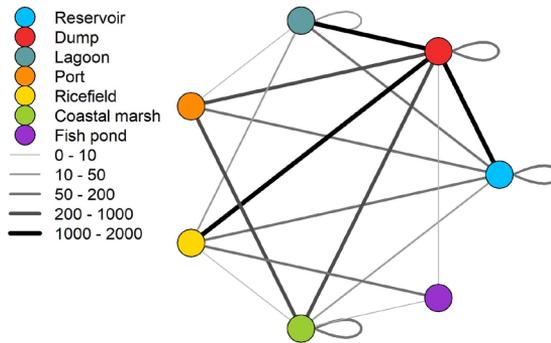


Figure 4. Overall connectivity between different habitat types in Andalusia. Thickness and colour intensity of the line reflect the number of direct flights between all polygons of a given habitat type. See Table S2 for precise numbers of direct flights.

The number of connections between nodes declined significantly with the distance between them ($R^2 = 0.635$, $t = -2.346$, $p = 0.020$), and increased with the number of tagged gulls connecting those nodes ($t = 12.314$, $p < 0.0001$). Long-distance connectivity between sites was rare, as only 2% (115 of 5,676) of the direct flights between nodes were beyond 60 km (Fig. S1). For example, there was just one direct flight between ricefields (DR) and Fuente de Piedra (FPL), the two sites where most gulls were counted.

Ten independent functional units (modules) were derived from the random walks algorithm, showing high rates of connectivity between the nodes within each unit, but low exchange between units (Fig. 3). Apart from unit 4 – which contained two coastal marsh sites – all other units contained at least one dump for foraging, and one natural or artificial wetland for roosting (Fig. 3). Functional units 3, 5, 6 and 7 in the central provinces of Seville, Málaga and Córdoba were the most important for maintaining the connectivity from West to East within the study area (Fig. 3).

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There were important temporal changes in the levels of connectivity within and between functional units during the course of the winter (Fig. 5). The number of flights was concentrated between December and February, peaking in January, and concentrated mostly within the functional units 3, 9 and 7, which were centred on ricefields, Fuente de Piedra and Jaen respectively. There was a steep decline in March, when the number of flights dropped within every functional unit as gulls began leaving their wintering quarters (Fig. 5).

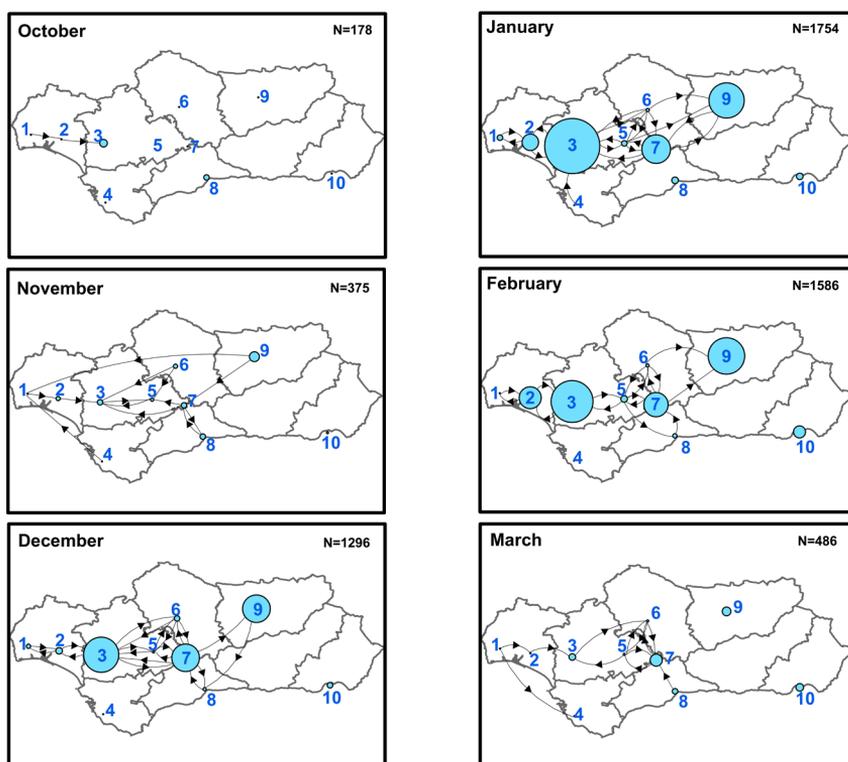


Figure 5. Temporal change of connectivity between the ten functional units during winter months in Andalusia (data from 2010 to 2017). Size of the circles reflects the relative number of directed flights performed within a given functional unit, whereas arrows reflect direct connections between functional units. N in the top right hand corner refers to the total number of direct flights that month. See Figure 2 for detailed information about functional units.

Discussion

Waterbirds can act as biological connectors between habitat patches, providing functional connectivity between terrestrial and aquatic habitats. We showed that LBBG is highly mobile and uses diverse habitats while wintering in southern Spain, thereby functionally connecting coastal, terrestrial and aquatic habitats. Our study is unique in identifying the connectivity network of a species over such a broad scale (87,268 km²). Functional connectivity for this species may be particularly high because it is, like most gull species, a generalist and opportunist that often travels long distances daily (**Chapter 4**; Thaxter et al., 2015). This functional connectivity can have major implications for the transport of nutrients and contaminants between sites, and for the dispersal of native and alien species able to survive gut passage, including pathogens (Lovas-Kiss et al., 2018a, see **Chapters 2 and 3**).

Importance of the Doñana ricefields

The use (in terms of time spent) of different habitats by LBBG varied during the winter season. Ricefields were the most used habitat during the first months of the winter (October-December), but their use gradually decreased after November, while the time spent in other habitats such as rubbish dumps, reservoirs, lagoons and coastal marshes increased. This was expected given the seasonal changes previously observed in LBBG censuses in ricefields (Rendón et al., 2008). Because harvesting and tilling occur in the first half of the winter, gulls can then exploit food sources such as the alien red swamp crayfish and spilled rice grains (Lovas-Kiss et al., 2018a; Toral et al., 2011).

The Doñana ricefields is the largest of our study polygons, and can provide both roosting and foraging habitats at the same time, e.g. feeding in a paddy that is being harvested whilst roosting elsewhere on a dyke or in a different paddy that has already been harvested (Guzmán et al., 1999; Toral et al., 2012). The high availability and predictability of resources during the rice harvest provides food for large numbers of gulls, but also allows them to be relatively stationary in the same general environment (Masero et al., 2011). Thus, only 15% of the visits to

the ricefields by gulls lasted more than 24 hours, but these represented 69% of the total time spent in the ricefields. Nevertheless, some of these long visits were also made during the second half of the winter. This indicates that some gulls were still feeding there after harvest was completed, perhaps on waste grain or invertebrates available in the fields that remained flooded.

In contrast to the decrease in time spent within ricefield habitat as the winter advances, the importance of gulls for functional connectivity between ricefields and other habitats increased in the transition to late winter. Connections between and within functional units varied seasonally, with a peak between December and February, when the role of gulls as biovectors may be especially important. The gulls then start moving between different habitats, likely due to decreasing food availability in the ricefields (Toral & Figuerola, 2010; Toral et al., 2011;), but may continue to use ricefields as a roosting site with daily visits to new foraging areas (Fujioka et al., 2010). These movements may also promote the spread of alien plant or animal species, which are particularly abundant in ricefields and whose propagules are dispersed by gulls at this time of year (Green, 2016; Lovas-Kiss et al., 2018a). Ricefields also contain many weeds, which often have herbicide-resistant genotypes, and gulls leaving ricefields are likely to disperse them to other suitable habitats (Farmer et al., 2017; Lovas-kiss et al., 2018a).

The key role of rubbish dumps in the connectivity network

If our only source of information about LBBG in Andalusia was the wintering waterbird census data from the wetland sites that are counted, we would have expected a high proportion of direct flights between those sites where most birds were counted (e.g. between the Doñana ricefields and Fuente de Piedra Lake). However, our movement network shows that this is not the case. The 12 rubbish dumps were identified as key sites in the network, although ten of them are not included in the waterbird census. Overall, 90% of direct flights between nodes were made to or from a dump, providing a direct connection between a terrestrial and aquatic habitat. Dumps provide easy and accessible resources without the need to expend much energy for searching (Duhem et al., 2005; Plaza & Lambertucci, 2017). For example, the rubbish dumps of Alcalá del Río (ARD)

and Alcalá de Guadaira (AGD) in the area surrounding the ricefields showed high connectivity with the ricefields themselves. Each functional unit contained complementary habitats, usually involving a major roosting site (reservoirs, lakes and other wetlands) and one or several foraging sites (ricefields, rubbish dumps or ports). Therefore, patterns of connections between rubbish dumps as foraging sites and wetlands as roosting sites are found along the connectivity network in most functional units (Fig. 3), and similar results were reported by GPS tracking of yellow-legged gulls *Larus michaellis* at a local scale within our unit 2 (Navarro et al., 2016). Although previous studies have shown the importance of the movement of marine nutrients onto land by birds (González-Bergonzoni et al., 2017; Irick et al., 2015; Sánchez-Piñero et al., 2000), our directed network shows that LBBG can transfer matter in the opposite direction: from inland rubbish dumps where they forage to coastal habitats where they roost, e.g. in functional units 2, 8 and 10 (Fig. 3).

Movement of gulls transporting nutrients into lakes and reservoirs from rubbish dumps occurs across North America, and causes important eutrophication effects (Winton & River, 2017). Such guantrophication is also a major process in Andalusia, and nutrient inputs by LBBG have been quantified for Fuente de Piedra (see **Chapter 4**). This current study shows the importance of many dumps in the same region, and how they are interconnected. Similarly, gulls using rubbish dumps can play an important role in biovector pathways of conventional and emerging contaminants (e.g. plastics, Persistent Organic Pollutants[POPs]) and heavy metals into natural ecosystems and into the human food chain (Desjardins et al., 2019; Kapelewska et al., 2019; Michielsen et al., 2018). LBBG wintering in Andalusia are known to carry a range of bacteria with Antimicrobial Resistance (AMR) genes (D. Jarma et al. unpublished data). As rubbish dumps are one of the most important sources for AMR (Arnold et al., 2016; Ramey et al., 2018), the direct movements of gulls between rubbish dumps and reservoirs, ricefields and ports may be of concern. This study provides a valuable step towards identifying specific pathways for AMR transmission by birds in Andalusia (see Arnold et al., 2016 for general AMR pathways).

Dispersal of plants and other organisms

Functional connectivity by LBBGs is mainly limited to within 60 km because only a small proportion of direct flights within Andalusia are longer. Nodes were aggregated in the functional units by proximity, which suggests connectivity was limited by distance, and connectivity between functional units was relatively low. Nevertheless, LBBG in Andalusia disperse many plants and invertebrates by endozoochory that would otherwise only be able to disperse over much shorter distances (Lovas-Kiss et al., 2018a). The connectivity network indicates that gulls make excellent vectors for stepping stone dispersal around Andalusia, in which novel organisms with a broad niche can be spread gradually around Andalusia by LBBG vectors. We also found that 7% of direct flights occur between different wetlands such as the ricefields, reservoirs, natural lakes and coastal marshes, and these connections may facilitate the dispersal of aquatic plants and invertebrates between localities, including alien bryozoans, snails and other invertebrates shown to survive gut passage by gulls (Lovas-Kiss et al. 2018a; see **Chapter 2**).

Implications for management

This study demonstrates how connectivity among wetland habitats can be indirectly stimulated by connections between aquatic and terrestrial habitats: because gulls are using rubbish dumps they are indirectly also increasing connectivity among inland wetlands. Our study further strengthens the notion that the growth in numbers of LBBGs on inland wetlands is the result of the expansion of rubbish dumps in Southern Spain, as previously reported for the UK (Harris, 1970). Long-term census data at Fuente de Piedra lake showed that LBBG have only become numerous in the past 30 years since landfills appeared (see **Chapter 4**). The expansion of ricefields in recent decades is also likely to have directly contributed to the increase of the wintering gull population, but our study shows that this is also partly because ricefields are a preferred roost site for gulls feeding at dumps.

This study provides a major insight into the likely pathways of biovectoring from

rubbish dumps. By identifying connections with key landfills, it can help to plan future studies of contaminant transport and develop management measures to reduce eutrophication and contamination issues, helping to protect biodiversity and water quality at key natural wetlands and reservoirs. A new National Framework Waste Management Plan (Plan Estatal Marco de Gestión de Residuos, 2015), based on the Landfill Management Directive, was approved in Spain in 2015 for the period 2016-2022. This directive requires the gradual reduction of biodegradable waste to 35% in 2016, with a further reduction of an additional 35% in 2020, as well as measures to improve waste separation and recycling. Such measures could potentially reduce the number of gulls at landfills and control the main pathways of terrestrial-aquatic connectivity and hence potential contamination.

This study demonstrates how gull movements provide important aquatic-terrestrial linkages. These are of great importance (Soininen et al., 2015), yet largely overlooked by the international community responsible for wintering waterbird censuses. There is great value in integrating studies of movement ecology with conventional waterbird surveys, because only the combination can provide a clear understanding of the connectivity between sites used by waterbirds. Based on movement data, key sites that lack survey data can be identified. Gull movement data suggests that counts at wetlands can be severely underestimated by missing birds that have flown to landfills (see **Chapter 4**). This study identified many important sites that are not covered by waterbird counts, which can help to improve future censuses. Our results suggest that the total number of LBBGs in Andalusia is likely to be much higher than that estimated from January counts (see Table 1).

Further work

Analyses like ours allow the scaling down of complex movements to a reduced set of nodes. Using a multi-state model framework, more specific covariates (e.g. age, sex or natal origin of gulls) could be incorporated in the future to identify key drivers of movement between nodes (e.g. Fremgen et al., 2017), and potentially also drivers of survival rates. Further studies should also address connectivity at

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different scales. Here we focused on connectivity at a regional scale, but focusing at more local scales may uncover new nodes and connections, and identify movements between other terrestrial and aquatic habitats of importance for biovectoring (e.g. the transport of weeds in agricultural land, or the transport of AMR into urban areas with risks for human health). On the other hand, network and connectivity analyses should also be applied to LBBGs in their breeding range. By assessing how stable such networks are through time, such analyses may help to identify critical breeding areas and enhance their conservation. Furthermore, the contribution of different LBBG individuals in connectivity networks should be investigated, as their roles may differ greatly. Specialist individuals may remain within a single functional unit throughout the winter, whereas more versatile, generalist individuals may have a more important role in both inter-habitat and long distance connectivity (between functional units).

Conclusions

LBBG provide important connections between terrestrial and aquatic habitats due to their high mobility and generalist behaviour. Doñana ricefields and various rubbish dumps performed as central nodes to maintain connectivity in the whole Andalusian region, although such connections changed seasonally together with changes in habitat use. High connectivity implies transport of organisms, nutrients, resistance genes and contaminants between different habitats by LBBG biovectors. Most transport occurs within 60 km distance and within ten functional units. This study has identified a unique multi-scale connectivity network between terrestrial and aquatic habitats, with important management implications.

Acknowledgements

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Table S2. Matrix of direct flights between the seven habitat types for the study period 2010-2017, n = 5,676 flights.

To From	Coastal marsh	Dump	Fish pond	Lagoon	Port	Reservoir	Ricefield
Coastal marsh	28	186	0	0	18	3	2
Dump	229	67	1	593	252	1150	822
Fish pond	0	0	0	0	0	0	62
Lagoon	0	623	0	30	2	13	13
Port	23	266	0	1	0	32	0
Reservoir	19	827	0	76	87	113	95
Ricefield	0	599	38	0	0	4	0

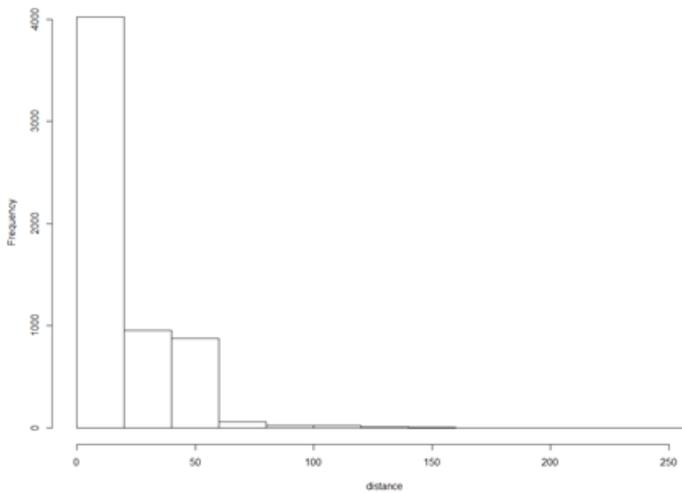


Figure S1. Frequency histogram of linear distances (in km) of direct flights between sites. Only 115 out of 5676 direct flights (2%) exceeded 60 km.

Chapter 2

Endozoochory of the same community of plants lacking fleshy fruits by storks and gulls



Martín-Vélez V., Lovas-Kiss Á., Sánchez M.I. & Green A.J.
Endozoochory of the same community of plants lacking fleshy fruits
by storks and gulls. In review in *Journal of Vegetation Science*.

Abstract

Research into the dispersal of plants lacking a fleshy fruit by avian endozoochory remains limited, particularly the different roles of specific vectors in the same habitat. We compared plants dispersed by endozoochory between two migratory waterbirds differing in body size: the lesser black-backed gull *Larus fuscus*, and the white stork *Ciconia ciconia*.

We collected faeces and pellets from roosting flocks on dykes in ricefields in Doñana, SW Spain, and extracted intact seeds. We recovered 424 intact seeds from excreta, representing 21 plant taxa, 11 of which germinated under laboratory conditions. Eight plant species are considered weeds, four of them as alien species, and only two have a fleshy fruit.

Seed abundance and species richness per sample were higher in storks than in gulls. Toadrush *Juncus bufonius* was the dominant species, accounting for 49% of seeds recovered. Permanova and mvabund analyses revealed no differences in the proportions of each plant species dispersed by the two vectors, and seasonal variation in abundance was absent. Overall, the germination rate was 18.9 %, and declined with increasing delay between sample collection and processing. Transects along dykes identified 52 plant taxa, only 18 of which were recorded in excreta.

Overlap in the communities of non-fleshy fruited plants dispersed by two unrelated birds of different size suggests that waterbird-plant dispersal networks are different from frugivore-plant networks. Unlike for frugivores, decoupling between seed production and ingestion reduces seasonal variation in endozoochory rates. For *J. bufonius* and other plants, these avian vectors provide maximum dispersal distances several orders of magnitude greater than predicted from their dispersal syndromes. Endozoochory by migratory waterbirds has major implications for plant distributions in a rapidly changing world, and more research is required before we can predict which plants disperse regularly via this mechanism.

Introduction

Dispersal is a crucial determinant of plant distribution, demography and genetic structure (Vekemans & Hardy, 2004; Caughlin et al., 2014), and therefore of plant responses to environmental perturbations, including anthropogenic land use change and climate change (Thuiller et al., 2008; Corlett & Westcott, 2013; Tamme et al., 2014). Moreover, dispersal ecology is central to the spread and potential control of alien plants and weeds (Gosper et al., 2005).

There are still knowledge gaps in our understanding of plant dispersal, including a need for a better characterization of dispersal vectors (Bullock et al., 2017). Many studies have relied on the classifications of floras into syndromes based on seed morphology to make predictions about vectors, and about dispersal distance (Thomson et al., 2010; Tamme et al., 2014). Animal vectors generally provide the longest dispersal distances for angiosperms (Bullock et al., 2017), yet dispersal syndromes assume that only plants with a fleshy fruit are dispersed by endozoochory (i.e. gut passage). However, repeated empirical studies have shown that this assumption is invalid (Costea et al., 2019; van Leeuwen et al., 2020).

Migratory waterbirds act as dispersal vectors for a broad variety of angiosperms (Green et al., 2016; Costea et al., 2019). In Europe, hundreds of non-fleshy fruited angiosperm species previously assigned to other syndromes have now been shown to be dispersed regularly by ducks and shorebirds via endozoochory (Soons et al., 2016; Lovas-Kiss et al., 2018b, 2019). Nevertheless, only a handful of detailed studies of waterbird endozoochory exist, compared to an extensive literature of plant dispersal by frugivorous birds (Wenny et al., 2016).

Consequently, basic questions remain unanswered, such as to whether there are specialised dispersal relationships between specific waterbird and plant species, or how the considerable range of body size and morphology amongst waterbird groups influences plant dispersal. It is well established that larger frugivores disperse plants with larger fruits (Jordano, 1995; Falcón et al., 2020) and that different bird species have different roles in plant-frugivore interactions

(Tsunamoto et al., 2020). In contrast, for non-fleshy fruited plants at a global scale, larger animals tend to ingest smaller seeds, and a higher number of plant species (Chen & Moles, 2015).

Over the past century, the extent of natural wetlands across the globe has been greatly reduced, whereas that of artificial environments such as ricefields has greatly increased (Davidson et al., 2018). Many waterbird species have shifted their habitat use and movement patterns to take advantage of agricultural environments such as ricefields, which are now important for waterbirds across the world (Rendón et al., 2008; Toral & Figuerola, 2010; Sesser et al., 2018). During the harvest period, food availability peaks and ricefields can support high numbers and diversity of waterbirds (Toral et al., 2011; Rendón et al., 2008; Sesser et al., 2018), which can disperse plants from the seed bank (Powers et al., 1978), which is particularly diverse for weeds (Chauhan et al., 2010). Within ricefields it is therefore possible to investigate the essential differences in seed dispersal between different plant vectors feeding in the same habitat.

Studies regarding the role of waterbirds as plant vectors within ricefields are scarce (Powers et al., 1978; Brochet et al., 2010). Waterbirds feed on the alien red swamp crayfish *Procambarus clarkii* in Iberian ricefields, and there is evidence for secondary dispersal of seeds carried on the outside of the crayfish by lesser black-backed gulls *Larus fuscus* (Lovas-Kiss et al., 2018a). The white stork *Ciconia ciconia* is a much larger waterbird also known to feed on crayfish within ricefields (Tablado et al., 2010), but its role in endozoochory is unknown. Both these species are benefitting from the expansion of artificial habitats (Rendón et al., 2008), and can show high functional connectivity between different habitat types (Chapter 1), increasing their potential as plant vectors.

In this study we compared endozoochory by these two omnivorous waterbirds in ricefields. We identified and quantified intact seeds through faecal and pellet analyses, and evaluated their germinability. Our specific objectives were: (1) To establish how plant dispersal interactions differ between these two different birds, and determine the roles of diet and seasonal variation. (2) To evaluate the traits and life history strategies of the plants dispersed, including whether

they were alien species or agricultural weeds. (3) To compare the plant species dispersed by these birds with the vegetation where seeds are egested, by carrying out transects along ricefield borders, and comparing traits between plants recorded and those dispersed.

Material and methods

Study area and study species

The ricefields of the Guadalquivir delta (37° 7' 50" -6° 9' 54", SW Spain, Fig. 1) are the largest ricefield region in Spain with 37 000 ha and are an important part of the Doñana wetland complex (Green et al., 2018). These ricefields support a diverse avifauna (Rendón et al., 2008; Toral & Figuerola, 2010). We selected two model species owing to their high abundance, major difference in morphology, and the ease with which their excreta could be collected from dykes.

The lesser black-backed gull (LBBG) is a wintering migratory waterbird breeding in North Europe (Baert et al., 2018). The white stork breeds from Northern Europe to West Africa with major differences in migration patterns between populations (Flack et al., 2016). Both species have increased across Europe and in the study area in recent decades (Wetlands International, 2020; Ramo et al., 2013; Rendón et al., 2008). The increases in numbers are related with increased food availability, largely from landfills and ricefields (Massemin-Challet et al., 2006; Ramo et al., 2013; see **Chapter 1**). Over 10,000 LBBG and over 1,000 white storks were present in the Doñana ricefields during our study. Mean body mass is 762 g for LBBG and 3,345 g for white stork (Wilman et al., 2014).

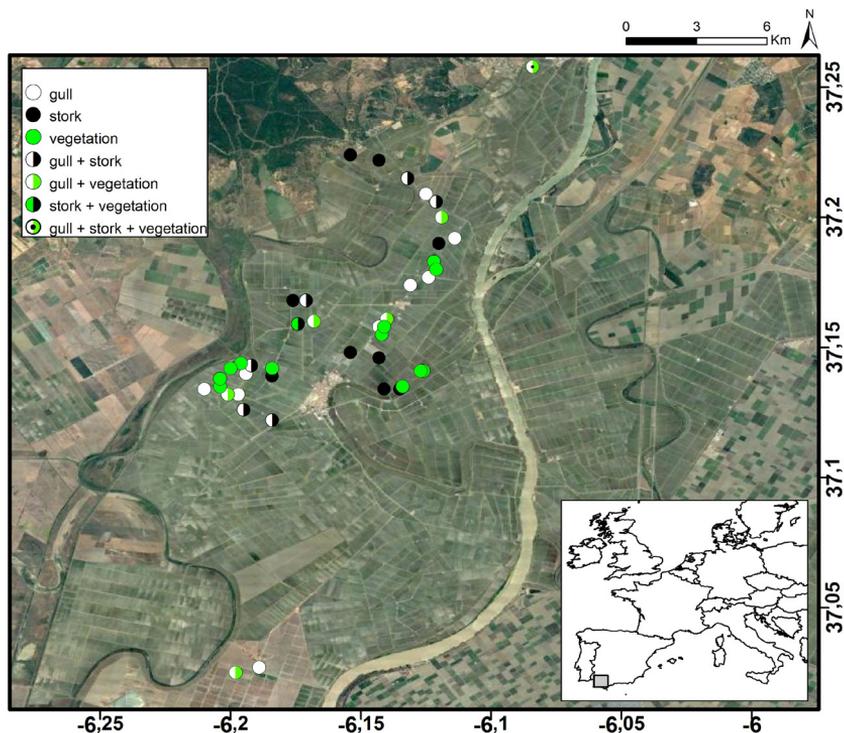


Figure 1. Location of sites within the ricefields of Doñana Biosphere Reserve where gull and stork samples (including pellets and faeces) were collected, and vegetation transects were monitored, during 2016 and 2017.

Excreta collection

A total of 463 excreta samples were collected in 36 different locations around the ricefields to the north-east of Doñana National park (Fig. 1). 183 samples (136 faeces and 47 pellets) were collected from white stork and 280 (183 faeces and 97 pellets) from LBBG during two consecutive winters: (1) November 2016 and (2) September, October and November 2017 (Table 1). Fresh faeces and pellets were collected from dykes that serve as field borders and public access routes, after visual confirmation of monospecific flocks that were resting on the dykes. Samples were taken from points separated by at least one-meter to ensure they were from different individuals. To avoid contamination, we removed the surface in contact with the soil with a knife before storing the samples in separate

zip bags. We preserved the samples in the fridge at 4°C until analysis. Average storage time was 35 days (range 4 to 80).

Table 1. Numbers and fresh mass in grams (reported median values and interquartile range (IQR) between brackets) of samples collected in ricefields from white stork and LBBG.

Species	Sample type	Median mass (IQR)	Nov. 2016	Sept. 2017	Oct. 2017	Nov. 2017
White stork	Faeces	2.55 (0.54)	51	32	23	30
	Pellets	8.0 (0.01)	22	-	14	11
LBBG	Faeces	2.0 (0.55)	53	42	47	41
	Pellets	8.2 (0.06)	25	15	27	30

Description of local flora along the dykes

Twenty vegetation transects were selected opportunistically to determine the most representative flora of the dykes across the ricefields (Fig. 1). Ten transects were carried out during February 2017 and ten during September-October 2017 in order to account for seasonal differences. We identified all taxa present in the transects along 100 meters in a straight line along the dykes, including moist soil plants at field edges. We did not sample vegetation within the ricefields, where gulls and storks were feeding, because we were unable to get permission to do so.

Sample processing

The fresh mass of pellet and faecal samples was first measured on a balance (Sartorius MSE225P) µg precision. Diet composition based on the main food items present was categorized as (1) crayfish based (2) rice based or (3) mixed (presence of both rice and crayfish). Samples were then sieved (100 µm mesh) and inspected under a stereomicroscope in Petri dishes. Plant diaspores (seeds and oogonia; “seeds” from hereon) were then retrieved, counted, photographed and measured (with ZEN 2-2.0 software). We identified to the lowest taxonomic

level by comparing the shape, size and seed coat pattern with available literature (Benedí & Orell, 1992; Bojnanský & Fargašová, 2007; Cappers et al., 2012; Castroviejo, 1998). When it was not possible to assign a morphotype to species level with certainty, genus or family level was reported instead. We did not include rice grains (*Oryza sativa*) as seeds dispersed because they were unlikely to be viable (Cummings et al., 2008). Immediately after retrieval, intact seeds were placed in Petri dishes that contained bacteriological agar and placed in germination chambers with 12/12 photoperiod and 22°C/18°C temperature conditions. Germination tests lasted for three months and seeds were checked every day for germination. Once germinated, seeds were counted and removed from the Petri dish. Seeds infected with fungi were removed from dishes to avoid cross-contamination, and considered not-germinated.

Statistical analyses

To evaluate the sampling effect on taxa richness within the samples, we carried out rarefaction analyses for each study species and sample type (Sanders, 1968), using the R package *iNEXT* for rarefaction analyses (Hsieh et al., 2016). We applied non-metric multidimensional scaling (NMDS) and Permanova analyses (applying Bray-Curtis for distance matrices) to identify differences in community composition between samples through the *metaNMDS* and *adonis* functions in *vegan* R package (Oksanen et al., 2010).

Abundance and richness (per sample) of seeds were compared between sample types (faeces or pellets), species (LBBG or white stork), period (November 2016, September 2017, October 2017 and November 2017) and diet (crayfish based, rice based or mixed) as fixed factors, using sample weight as a continuous variable and sampling location as a random factor. We used Generalized Mixed Models (GLMM) with negative binomial error distribution and log link function under the *glmmTMB* package (Magnusson et al., 2017) to account for the many samples with zero values and overdispersion. For the dominant species *Juncus bufonius*, we carried out similar GLMM analyses for abundance, but results were the same as for total seed abundance (details not shown). For *J. bufonius*, we also tested the effect of bird species, sample type, diet, period and storage time

on germinability (binomial model) and time (days) until germination (linear model) with the package *lme4* (Bates et al., 2014).

We carried out multivariate negative binomial tests for abundance of the remaining taxa, which was much lower than for *J. bufonius*. These tests included the variables species, sample type, period and diet and were performed with the `manyglm` function in *mvabund* package (Wang et al., 2012). All analyses were performed with R (v3.3.4 R Core Team, 2018).

We calculated the Jaccard Index (JI) to compare similarities in species composition between excreta samples and vegetation transects (details in supplementary material). We assigned to every taxa found in excreta and/or transects a mean seed weight (from LEDA traitbase; Kleyer et al., 2008), a dispersal syndrome (from baseflor, Julve, 1998) and an Ellenberg F value (Julve, 1998; Hill et al., 1999). F indicates plant soil moisture preference, and varies from one to 12 (e.g. a value of one indicates extremely dry soils, whereas 9 indicates wet soils). Finally, to evaluate potential determinants of relative abundance of different taxa within excreta, we tested if abundance was related to seed length or mass (through correlations) and dispersal syndrome (via a `kruskal-wallis` test, with `dunn` test for posthoc; *dunn.test* R package, Dinno & Dinno, 2017), or related to the frequency of plants along dykes by correlating with % occurrence within transects.

Results

Mass and general content of bird excreta

On average excreta samples from storks were heavier than those from gulls (Table 1). These differences were significant for faeces ($U= 15015$, $p = 0.002$), but not for pellets ($U= 2145$, $p = 0.568$). Crayfish remains were recorded in 79% of stork and 70% of gull pellets, compared to 93% of stork and 78% of gull faecal samples. Rice grains were the next most prevalent food item, and were often combined with crayfish remains. Rice was present in 28% of stork and 43% of gull pellets, compared to 24% of stork and 28% of gull faecal samples.

Plant seeds recovered from bird excreta

Overall, 35% (165 of 464) of excreta samples contained at least one intact seed, and 424 intact seeds from 21 different plant taxa were recorded (Table 2). These included a range of terrestrial, moist soil, and aquatic species, assigned to six different dispersal syndromes and 11 Ellenberg moisture categories (Table S1, Fig. 2). Eight (38%) of these 21 taxa are agricultural weeds, and four (18%) are alien species in Spain (Table 2).

More specifically, 59% of stork pellets contained at least one intact seed, and 45% of stork faeces, compared to 23% of gull pellets and 29% of faeces (Table 2). Nineteen taxa were recorded in stork samples and only 12 in gulls, with ten taxa (48% of the total) recorded in both vector species, nine only in storks and two only in gulls (Table 2). The plant community dispersed did not differ significantly between vector species for either pellets (Permanova; $F_{21} = 1.18$; $p = 0.310$) or faeces (Permanova; $F_{21} = 0.83$, $p = 0.405$) (Table 2, Fig. 3). *Juncus bufonius* was the most abundant taxon in all sample types, representing 49% of all intact seeds (Table 2). Mean seed length per taxon ranged from 0.4 mm (*Juncus subnodulosus*) to 1.85 mm (*Solanum nigrum*). Mean seed length per sample did not vary significantly between bird species ($U = 3239$, $p = 0.819$) or sample type ($U = 2631$, $p = 0.826$). Mean seed mass and mean length for a given taxon were significantly correlated ($n = 14$, $r_s = 0.79$; $p < 0.001$). Total abundance of seeds of a given taxon within all excreta samples was significantly correlated with mean mass ($n=14$, $r_s = -0.73$, $p = 0.003$) but not mean length ($n = 21$, $r_s = -0.26$, $p = 0.253$). There were significant differences in the number of seeds from each dispersal syndrome in a given sample ($H = 350.22$, $df = 5463$, $p < 0.001$). This was due to significantly greater abundance for epizoochory (to which *J. bufonius* was assigned) than for other syndromes (Fig. 2).

Table 2. Details of intact seeds found in white stork and LBBG excreta samples from the winters of 2016-2017. Shown are whether or not a taxon was recorded within dyke transects (dyke occur.), total number of seeds found in excreta, the number of samples in which each taxon was recorded (NST), the combined number of seeds in those samples (N seeds) and the maximum number of seeds recorded in a single sample (Max. sample).

Family	Taxa	Dyke Occur	Total	<i>Larus fuscus</i>				<i>Ciconia ciconia</i>						
				Pellets (N=97)		Faeces (N=183)		Pellets (N=47)		Faeces (N=136)				
				NST	N seed	Max. sample	NST	N seed	Max. sample	NST	N seed	Max. sample		
Amaranthaceae	<i>Amaranthus albus</i> ^{a, b, c}	Yes	10	1	1	1	1	3	3	4	4	1	2	2
	<i>Amaranthus retroflexus</i> ^{a, b, c}	No	1	-	-	-	-	-	-	1	1	1	-	-
Asteraceae	<i>Lemna minor</i>	Yes	3	1	1	1	-	-	-	-	-	-	1	2
	<i>Lemna gibba</i>	Yes	2	-	-	-	-	-	-	1	1	1	1	1
Caryophyllaceae	<i>Spergularia maritima</i>	Yes	27	-	-	-	-	-	-	1	24	24	1	1
Characeae	<i>Chara</i> sp.	-	35	3	3	1	9	11	3	3	12	9	8	9
		-	2	-	-	-	-	-	-	-	-	-	1	2
Cyperaceae	<i>Chenopodium</i> cf. <i>album</i>	-	38	2	2	1	8	11	3	7	10	4	10	15
Elatinaceae	<i>Cyperus difformis</i> ^{b, c}	No	1	-	-	-	1	1	1	-	-	-	-	-
	<i>Bergia capensis</i> ^a	No	1	-	-	-	-	-	-	-	-	-	-	-
Fabaceae	<i>Trifolium repens</i>	Yes	1	-	-	-	-	-	-	1	1	1	-	-
	<i>Juncus subnodulosus</i>	Yes	15	1	1	1	3	3	3	4	5	2	5	6
Juncaceae	<i>Juncus infolatus</i> ^b	Yes	210	14	20	4	35	52	4	20	42	10	45	96
	<i>Polygonum monspeliensis</i> ^{b, c}	Yes	2	-	-	-	-	-	-	-	-	-	2	2
Poaceae	<i>Sorghum</i> cf. <i>indipense</i> ^{a, b, c}	No	2	-	-	-	-	-	-	1	2	2	-	-
	<i>Panicum</i> cf. <i>miticatum</i>	No	1	-	-	-	-	-	-	1	1	1	-	-
Portulacaceae	Unidentified	-	2	-	-	-	1	1	1	1	1	1	-	-
	<i>Portulaca oleracea</i> ^{b, c}	Yes	4	1	1	1	1	1	1	1	1	1	1	2
Ranunculaceae	<i>Ranunculus sceleratus</i>	Yes	34	1	1	1	8	9	2	4	5	2	9	19
	<i>Ranunculus repens</i>	Yes	1	-	-	-	-	-	-	1	1	1	-	-
Solanaceae	<i>Solanum nigram</i> ^{b, c}	Yes	14	1	5	5	2	6	3	-	-	-	-	-
	<i>Solanum dulcamara</i>	Yes	19	1	1	1	2	15	8	1	1	1	3	3
Total			424	26	36	5	70	112	8	52	111	10	88	158

^a Taxa alien to Spain according to AgroAtlas (2005), <http://agroatlas.ru>, ^b considered an agricultural weed, and ^c known to have herbicide resistant populations according to Heap (2009).

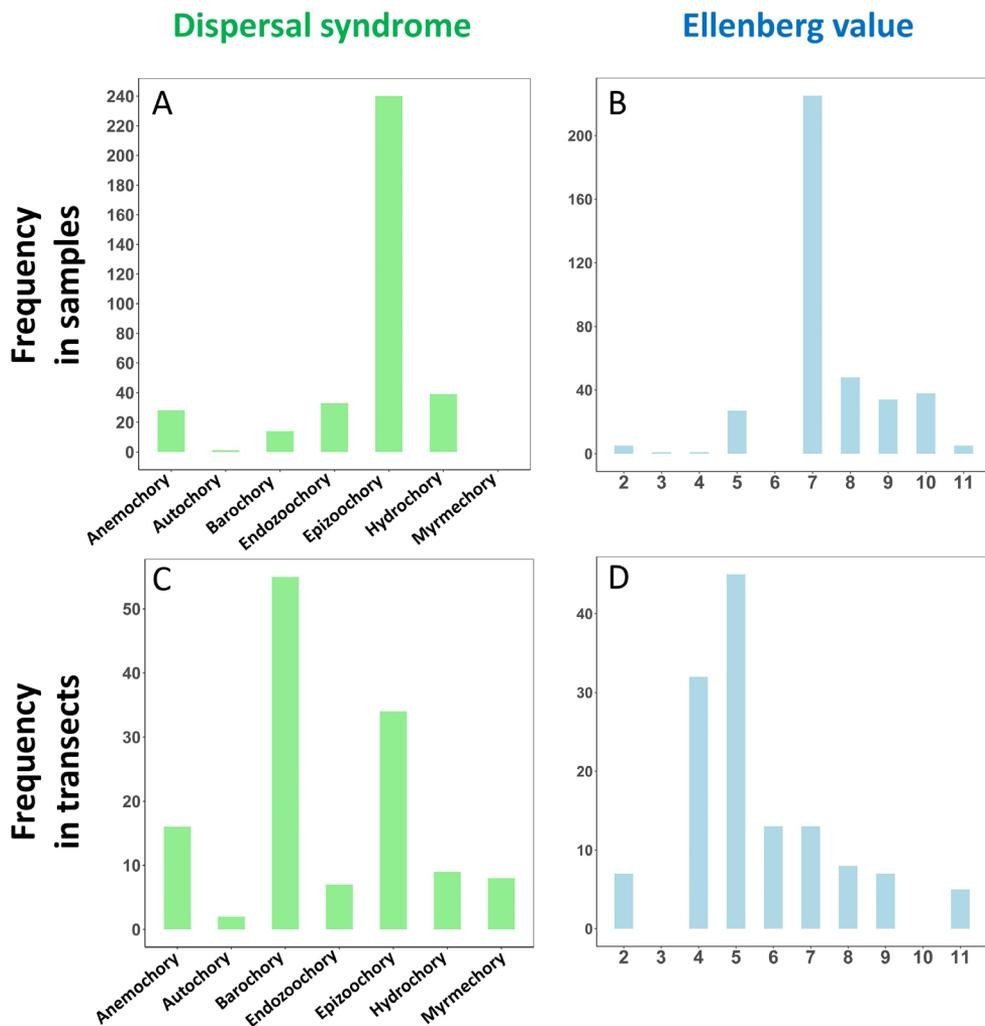


Figure 2. Frequency distributions of dispersal syndromes and Ellenberg moisture values based on the abundance of different species. A) and B) are for seeds from gull and stork excreta. C) and D) are based on the frequency of occurrence in 20 vegetation transects. *Juncus bufonius* (the dominant taxon in excreta) has an epizoochory syndrome and an Ellenberg value of 7.

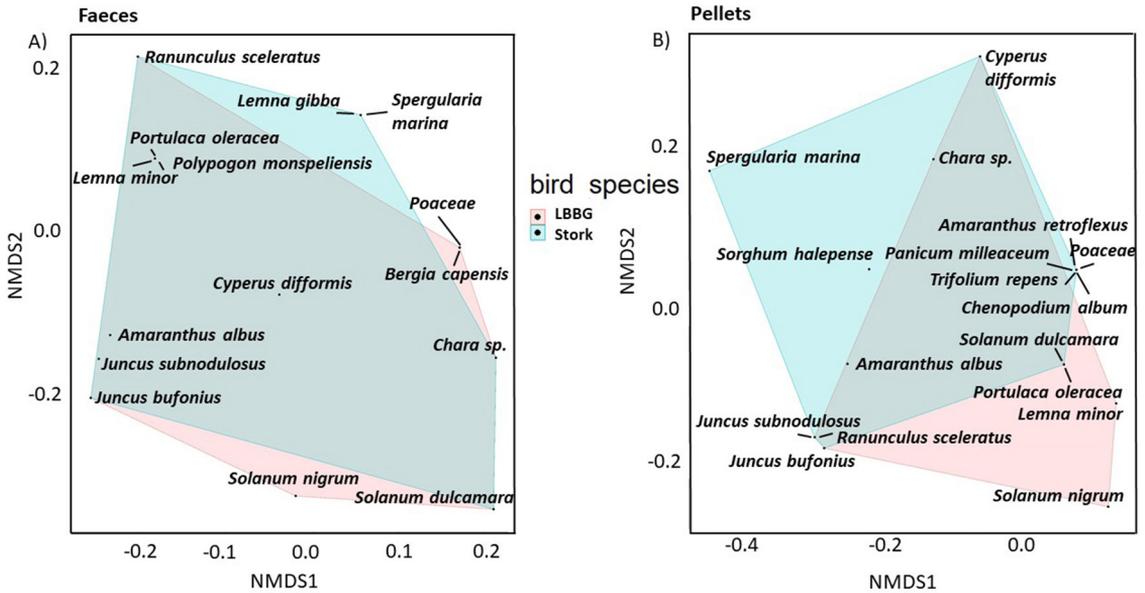


Figure 3. Non-metric multidimensional scaling (NMDS) plot showing the relationship between seeds dispersed by LBBG and white stork in faeces (A) and pellets (B) in ricefields.

Mixed models showed that bird species, sample type and sample mass all had significant partial effects on the total abundance of seeds in samples, as well as on the species richness (Table 3). Neither abundance nor species richness were significantly influenced by sampling period or the relative content of rice and crayfish in samples (Table 3). Gulls and pellets had significantly fewer seeds and fewer plant taxa per gram of excreta (Table 3). When sample mass was removed as a predictor from the models, pellets no longer had significantly more seeds ($\chi^2 = 0.085$, $p = 0.77$) or taxa ($\chi^2 = 0.254$, $p = 0.61$) than faecal samples. However, storks still had significantly more seeds and plant taxa per sample than gulls ($\chi^2 = 19.6$, $\chi^2 = 20.9$, respectively; $p < 0.001$).

Table 3. Effects of bird species, period, diet, sample type and weight on (A) total abundance of seeds and (B) taxon richness per sample, from negative binomial mixed models. Samples from White stork, faecal samples, samples from November 2016, and those with a diet of crayfish were aliased, and so effectively had estimates of zero. Sampling location (Figure 1) was included as a random factor. Shown for each term are the parameter estimates (β) and their standard errors, and the main effects for each predictor variable.

(A)	Seed abundance	Level of effect	β	S.E.	χ^2	<i>p</i>
	Species	LBBG	-0.726	0.183	15.738	<0.001
	Period	Sept. 2017	0.11	0.322	4.127	0.248
		Oct. 2017	-0.048	0.327		
		Nov. 2017	0.546	0.31		
	Diet	Mixed	0.733	0.252	2.069	0.355
		Rice	0.338	0.238		
	Sample mass		0.076	0.014	30.31	<0.001
	Sample type	Pellets	-0.594	0.219	7.393	0.007
Random contribution (variance): location= 0.156						
(B)	Plant richness	Level of effect	β	S.E.	χ^2	<i>p</i>
	Species	LBBG	-0.647	0.168	14.784	<0.001
	Period	Sep. 17	0.016	0.281	4.751	0.191
		Oct. 17	-0.167	0.286		
		Nov. 17	0.444	0.264		
	Diet	Mixed	0.096	0.233	0.586	0.746
		Rice	0.164	0.22		
	Sample mass		0.069	0.012	30.312	<0.001
	Sample type	Pellets	-0.459	0.203	5.108	0.024
Random contribution (variance): location= 0.0908						

Rarefaction curves revealed steeper slopes for species richness against sample size for storks than for gulls, particularly for pellets, suggesting that stork pellets contained a higher diversity of seeds (Fig. 4). Nevertheless, seed composition analyses with *mvabund* showed that no plant species was significantly associated with one vector, nor with faeces or pellets (Table S2). The only significant effects were seasonal, the probability of finding *Ranunculus sceleratus* and *Cyperus difformis* seeds being particularly high in November 2017 (Table S2).

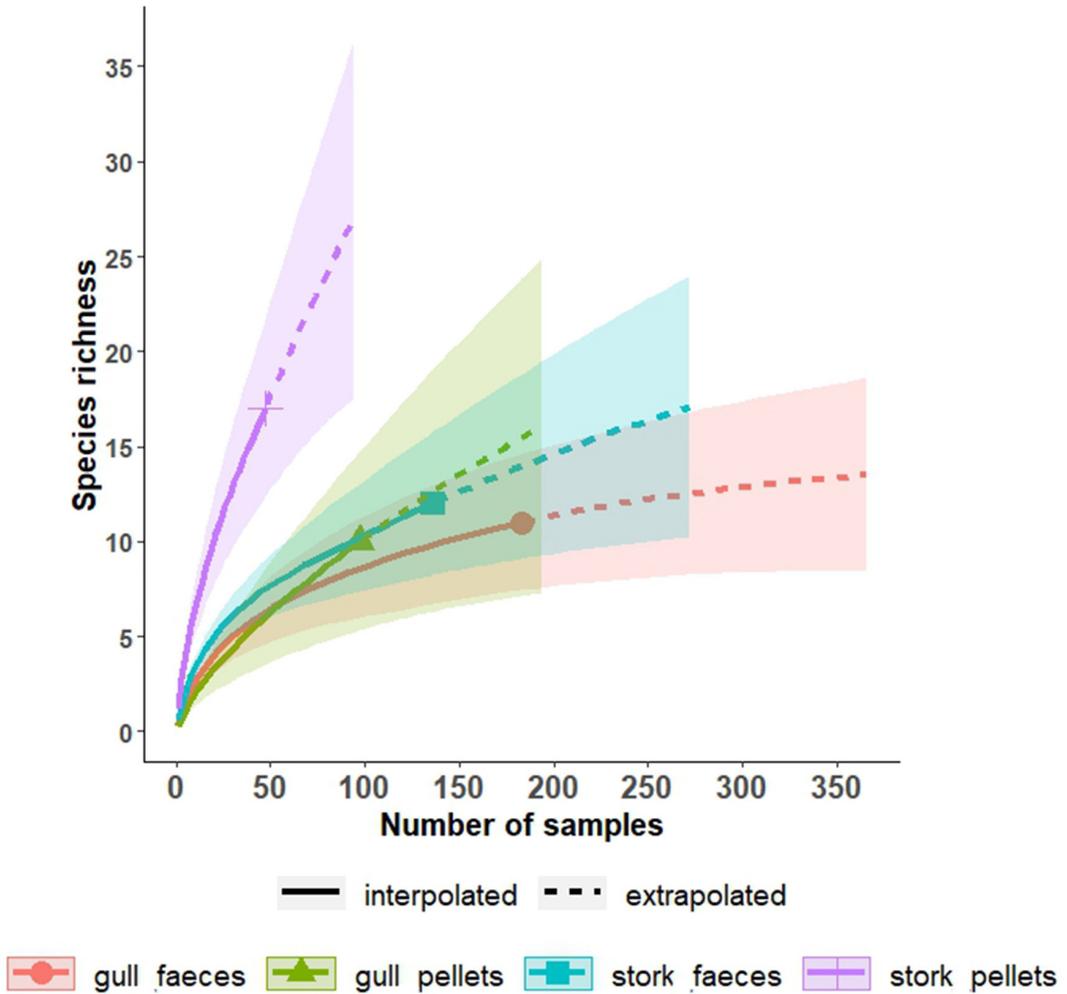


Figure 4. Rarefaction analyses showing the accumulated number of plant taxa recorded in pellets and faeces of white storks and LBBG, in relation to the number of samples. Error bars represent 95% Confidence Intervals.

Table 4. Germination of seeds found in LBBG and white stork pellets and faeces.

Plant family	Plant taxa	Total diaspores	Total germinated	<i>Larus fuscus</i>				<i>Ciconia ciconia</i>			
				Pellets		Faeces		Pellets		Faeces	
				N diaspores	N germinated	N diaspores	N germinated	N diaspores	N germinated	N diaspores	N germinated
Amaranthaceae	<i>Amaranthus albus</i>	9	6	1	-	3	3	3	1	2	2
	<i>Amaranthus retroflexus</i> ^{1,2,3}	1	1	-	-	-	-	1	1	-	-
	<i>Lemna minor</i>	3	0	1	-	-	-	-	-	2	-
Caryophyllaceae	<i>Lemna gibba</i>	2	0	-	-	-	-	1	-	1	-
	<i>Spergularia marina</i>	27	11	-	-	-	-	24	11	1	-
Characeae	<i>Chara sp.</i>	35	0	3	-	11	-	12	-	9	-
	<i>Chenopodium cf. album</i> ^{1,3}	3	3	-	-	-	-	1	1	2	2
Cyperaceae	<i>Cyperus difformis</i> ¹	38	4	2	1	11	-	10	2	15	1
	<i>Bergia capensis</i>	1	0	-	-	1	-	-	-	-	-
Elatinaceae	<i>Bergia capensis</i> ^{1,3}	1	1	-	-	-	-	1	1	-	-
	<i>Trifolium repens</i>	1	1	-	-	-	-	1	1	-	-
Juncaceae	<i>Juncus subnodulosus</i> ¹	15	1	-	-	-	-	1	-	-	1
	<i>Juncus bufonius</i> ^{1,2,3}	210	39	20	4	52	12	42	4	96	19
Poaceae	<i>Polygonum monspeliensis</i> ¹	2	0	-	-	-	-	-	-	2	-
	<i>Sorghum cf. halepense</i>	2	0	-	-	-	-	2	-	-	-
	<i>Panicum cf. milleanum</i>	1	0	-	-	-	-	1	-	-	-
	<i>Unidentified</i>	2	0	-	-	1	-	1	-	-	-
Portulacaceae	<i>Portulaca oleraceae</i>	4	2	1	-	-	-	1	1	2	1
	<i>Ranunculus sceleratus</i> ^{1,2}	34	0	1	-	9	-	5	-	19	-
Ranunculaceae	<i>Ranunculus repens</i> ³	1	0	-	-	-	-	1	-	-	-
	<i>Solanum nigrum</i> ¹	14	2	5	-	6	2	-	-	-	-
Solanaceae	<i>Solanum nigrum</i> ¹	19	10	1	-	15	10	1	-	3	-
	<i>Solanum dulcamara</i> ¹	1	1	-	-	-	-	-	-	-	-
Total		424	80	35	5	110	27	108	22	154	26

¹endozoochory previously reported for intact seeds in gulls (Calvino-Cancela 2011; Lovas-Kiss et al., 2018b).

²germination after gut passage confirmed in Lovas-Kiss et al., 2018b.

³ seed dispersal previously reported within white stork nest material (Czarnecka & Kitowski, 2013).

Germinability of diaspores from excreta

Overall, germination was recorded for 11 (52%) of taxa, with an overall germination rate of 18.9 % (Table 4). For the dominant *J. bufonius* 19.5% of seeds germinated, and germinability was significantly affected both by sampling period and the time that excreta samples were stored in the refrigerator before processing (Table 5). Viability was significantly lower in October 2017 than in November 2016 (Post-hoc test, $Z = -2.076$, $p = 0.038$). The time taken for *J. bufonius* to germinate was also significantly affected by sampling period (Table 5). Germination time was significantly longer in September 2017 than in November 2016 ($Z = 2.436$, $p = 0.022$).

Table 5. Effects of species, period, sample type, diet and storage time on germinability (A) and germination time (B) of *Juncus bufonius* based on binomial and linear models respectively. Samples from LBBG, faecal samples, samples from November 2016, and those with a diet of crayfish were aliased. See Table 3 for further explanation.

(A)	Germinability <i>Juncus bufonius</i>	Level of effect	β	S.E.	χ^2	p
	Species	White stork	-0.493	0.443	0.074	0.786
	Period	Sept. 2017	-0.213	0.56	8.416	0.038
		Oct. 2017	-2.304	1.11		
		Nov. 2017	-0.226	0.548		
	Diet	Mixed	-0.434	0.703	1.319	0.517
		Rice	-0.167	0.564		
	Sample type	Pellets	-0.528	0.4644	2.405	0.121
	Storage time		-0.024	0.013	3.893	0.048
(B)	Germination time <i>Juncus bufonius</i>	Level of effect	β	S.E.	χ^2	p
	Species	White stork	-0.196	1.114	0.098	0.756
	Period	Sep. 2017	3.4923	1.434	3.522	0.028
		Oct. 2017	-1.621	3.204		
		Nov. 2017	2.325	1.587		
	Diet	Mixed	-2.262	2.118	1.002	0.38
		Rice	-1.487	1.512		
	Sample type	Pellets	0.672	1.428	0.048	0.827
	Storage time		-0.001	0.043	0.006	0.938

Adj. $R^2=0.119$.

Relationship with vegetation along dykes

A total of 52 plant taxa were recorded in 20 vegetation transects (Table S2). Overall, 13 species of these taxa (26%) were recorded in excreta (Table 2). Jaccard Index values showed limited similarity between species recorded in transects and excreta (0.19 for LBBG and 0.25 for storks). *Juncus bufonius* and *Conyza canadiensis* were the taxa recorded most often within transects, and the latter was absent from excreta (Table S2). Five species present in excreta were not detected in dyke transects (Table 2 and Table S2), including *Cyperus difformis*, a tall weed abundant within rice stands. There was no correlation between total abundance of seeds per taxon in excreta samples and its frequency of occurrence within transects ($N = 48$, $r_s = -0.15$, $p = 0.298$). Neither was there any difference in seed mass between taxa unique to transects, unique to excreta, or found in both ($H = 4.95$, $df = 2$, $p = 0.08$).

Comparisons of syndromes between seeds in excreta and plants in transects (Fig. 2) show that epizoochory syndrome is overrepresented in excreta (representing 68 % of seeds), due to the dominance of *J. bufonius*. In contrast, the barochory syndrome (4% of seeds) is underrepresented in excreta, and only 9% of seeds had an endozoochory syndrome. Comparing Ellenberg moisture values between excreta and transects (Fig. 2) suggests that 7 (moist soils) is overrepresented in excreta, again due to the dominance of *J. bufonius*. Transects are dominated by dry soil plants with an Ellenberg value of 2 to 6 (77% of all plant records), uncommon values in excreta (19.5% of all seeds).

Discussion

We studied the plant taxa dispersed by a gull and a stork species through endozoochory in an agricultural landscape during three months of the migration and overwintering period. The seeds quantified were dispersed from feeding sites within ricefields to dykes where birds roosted. Most seeds dispersed lacked the fleshy fruit classically linked to avian endozoochory. Our findings add to growing evidence that endozoochory of non-fleshy fruited plants (“non-classical endozoochory”; Costea et al., 2019) by migratory birds is a major ecological

process. Our results for storks extend the list of waterbird groups shown to be important vectors for endozoochory, adding to shorebirds, Anatidae and others (Green et al., 2016). The consistency between our results and those for gulls in previous studies (Lovas-Kiss et al., 2018a; Calvino-Cancela, 2011) illustrates how “non-classical endozoochory” can be a predictable process comparable to endozoochory by frugivores, including the dominance of *J. bufonius* in ricefields.

On the other hand, we recorded seed dispersal of eight species not previously recorded in gulls or storks, including three alien species *Amaranthus albus*, *Bergia capensis*, and *Sorghum halepense*. Many of these new taxa were recorded in small numbers, and their detection was subject to sampling error, as illustrated by rarefaction (Fig. 4). Therefore, the apparent differences we recorded in the species dispersed by each vector may be purely a result of sampling error, and the number of plant taxa dispersed by the stork and gull populations may be much higher than that detected, possibly including all the plants detected in our transects. We confirmed that 52% of the plant taxa found can germinate after gut passage, and this is an underestimate given the small sample size ($N \leq 3$) of the angiosperm species that failed to germinate (Table 4, *Ranunculus sceleratus* was an exception).

Lovas-Kiss et al. (2018a) found evidence that seeds dispersed by LBBG within ricefields were ingested involuntarily when feeding on crayfish, which have small seeds stuck on the outside. Given the small size of the seeds we recorded and the negative correlation between abundance and seed mass, it seems unlikely that gulls or storks would be actively foraging on them. However, diet content (rice versus crayfish) did not influence the abundance and richness of seeds in our samples, suggesting that birds also ingest seeds when feeding on rice within the mud of harvested fields. The *Solanum* spp. we recorded are likely to be an exception, as these plants grow along the dykes and have berries that are likely to be ingested actively, especially by gulls (Calvino-Cancela, 2011).

Egestion via faeces represents the main form of endozoochory in our study system. Storks produce four times more faeces than pellets in dry mass per day (Kwieciński et al., 2006). Faeces are also egested in a greater diversity of

microhabitats, including feeding and roost sites as well as during flight, whereas pellets are only likely to be egested in roosting places including dykes.

Differences between storks and gulls as vectors

Plant community analyses did not detect overall differences between the two bird species. This suggests a high degree of functional redundancy in their role as vectors, although there are differences in their movement and migration patterns (e.g. only storks breed in the study region). In our case, avian body mass was not a trait determining dispersal interactions, in contrast to frugivore studies (Costa-Pereira et al., 2018; Chen & Moles, 2015). Storks weigh four times more than gulls and have a much wider gape, yet we found no difference in the size of seeds dispersed. Mean seed length of the plant taxa dispersed was 0.86 mm (\pm 0.08 S.E., range = 0.4-1.85 mm), showing a strong representation of small seeds. Taxa with relatively smaller and relatively harder seeds have higher survival during avian gut passage (Reynolds & Cumming, 2016; Lovas-Kiss et al., 2020). Although harder and/or large food items (including large seeds) are more likely to be egested in pellets than in faeces (Sánchez et al., 2005; Lovas-Kiss et al., 2019), we found no difference in seed size between these two forms of excreta, presumably owing to the generally small size of the seeds.

The larger size of storks may directly have influenced the proportion and distance of seeds dispersed following ingestion. Differences in waterbird body size and diet have previously been shown to influence seed survival and retention time within the gut, and hence the probability and distance of seed dispersal (Reynolds & Cumming, 2016; Viana et al., 2013).

We found greater abundance and richness of seeds per sample, or per gram of excreta, in storks than in gulls. Despite the lower number of samples processed for storks, the higher number of seeds per sample increased the chances of finding rare species, and thus the total taxa richness. Per individual, the larger storks ingest more, egest more, and disperse more seeds a day than gulls. However, LBBGs are about ten times more abundant than white storks within the ricefields. Bearing in mind the peak numbers of gulls and storks counted

(Estación Biológica de Doñana monitoring data), and estimates for daily production of excreta (see **Chapter 4**), in the order of 10^5 intact seeds per day (half being of *J. bufonius*) are dispersed within the ricefields by these two bird species alone at peak periods.

The lack of difference between storks and gulls in plants dispersed suggests that other birds of an intermediate size (e.g., herons, egrets, black storks, glossy ibis, other gulls) that are abundant in ricefields and feed in a similar manner on crayfish (Tablado et al., 2010) may be vectors for the same plant species. On the other hand, other birds such as ducks, shorebirds and greater flamingos have different feeding strategies, and may disperse plants represented in ricefield seed banks in different proportions.

Comparison between seeds dispersed and vegetation transects

Endozoochory by storks or gulls was only recorded for 26% of plant taxa from transects along dykes. Most of the plant taxa in excreta have high water requirements (Fig. 2) and occur within rice stands, but not along the dykes. Vegetation transects were conducted along the dyke habitats where birds roosted, and thus recorded plants with low moisture requirements (e.g. *C. canadensis*, absent from excreta) on the top of dykes (Fig. 2) and generalist plants occurring at field edges. However, 72.6% of seeds from excreta were from taxa recorded in transects, therefore seeds dispersed to dykes by birds may become established.

Previous comparisons between plant taxa dispersed by ducks or geese, and those that are not, indicates that plant species with small seeds are more likely to disperse by waterbird endozoochory (Soons et al., 2016; Hattermann et al., 2019). This is consistent with our result that, amongst species recorded in excreta, those with smaller seeds were more abundant. In our comparison with the flora of dykes, we found no evidence that small seeds are favoured for endozoochory, but these disturbed habitats are themselves dominated by ruderal species likely to have small seeds.

Seasonality

Different management practices (e.g. harvesting, tilling) may change the availability of different species in the seed bank and of food resources for birds in ricefields (Toral et al., 2011; Li et al., 2012; Chauhan et al., 2006), but we recorded no seasonal variation in the species richness and abundance of seeds dispersed by birds. The only seasonal effects were for *Ranunculus sceleratus* and *Cyperus difformis*, which showed less abundance in September and October respectively. Both species grow within the rice stands, and perhaps their seed dispersal may be favoured by tilling practices in November. Since storks and gulls are generally dispersing seeds after they have left the mother plant and have entered the seed bank, this decoupling between seed maturity and endozoochory means that differences in phenology between plant species dispersed does not readily translate into differences in the timing of dispersal. Similarly, Brochet et al., (2010) found no seasonal changes in the frequency of endozoochory in teal *Anas crecca* wintering in the Camargue and feeding partly in ricefields. In contrast, frugivorous birds can show major temporal differences in the proportions of different plants dispersed (Carnicer et al., 2009; Vázquez et al., 2009).

Possibly, much greater variation between months and bird species in plants dispersed would be recorded if excreta were sampled within natural wetlands. Ricefields are more predictable in their flooding patterns and food resources than nearby natural wetlands in Doñana, and there are important differences in the bird communities they hold (Rendón et al., 2008; Sebastián-González & Green, 2016).

Importance of endozoochory for long-distance dispersal

Storks, gulls and other birds in ricefields regularly move into natural wetlands as well as into different agricultural habitats, facilitating the dispersal of alien species, weeds and other plants between habitats (Rendón et al. 2008; Ramo et al. 2013; **Chapter 1**). The plant species dispersed in our study are found

in many other natural and anthropogenic habitats apart from ricefields. For example, *J. bufonius* occurs in various terrestrial habitats such as grasslands (Milotic & Hoffmann, 2016), is a new alien species in extreme latitudes (Cuba-Diaz et al., 2013), and may provide an interesting model for the study of how zoochory influences genetic patterns at different spatial scales. Many of the plants we recorded in excreta samples are agricultural weeds, some of which have herbicide resistant populations (Table 2), and waterbirds may facilitate their effective dispersal to other habitats beyond ricefields (Farmer et al., 2017).

Interestingly, five species dispersed in our study were previously reported for storks in Polish agricultural landscapes (Czarnecka & Kitowski, 2013). This degree of overlap is surprising, given that the Polish study was of seed dispersal by storks into their nests (the mechanism is unclear, and could be transfer in the beak as nest material, or via excreta) in a region lacking ricefields and with a different climate. It suggests there is a class of non-fleshy fruited plants (e.g. *J. bufonius*, *Amaranthus retroflexus*), with an extreme ability to disperse via birds, which is worthy of future research.

Tamme et al. (2014) considered *J. bufonius* to have a maximum dispersal distance of 100 m (via wind dispersal). Similarly, they considered *R. sceleratus* to have a maximum dispersal distance of 35 m and *Spergularia marina* one of 340 m (via wind). All three of these species are dispersed by storks and gulls over much longer distances, illustrating how studies that make macroecological predictions about plant dispersal based on syndromes (e.g. Thomson et al., 2010; Tamme et al., 2014) are likely to be unreliable, given the consequences of ignoring non-classical endozoochory. *Juncus bufonius* is also dispersed by endozoochory by shorebirds (Lovas-Kiss et al., 2019), at least five species of Anatidae (Lovas Kiss et al., unpublished) and ungulates (Milotic & Hoffmann, 2016). Different authors assigned to this taxon to anemochory, hydrochory and epizoochory syndromes (Löve, 1963; Cope & Stace, 1978; Julve, 1998), exemplifying the subjectivity when syndromes are assigned based on seed morphology, without adequate field data on dispersal mechanisms.

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Numbers of both storks and LBBG have increased in SW Spain in recent decades (Rendón et al., 2008; Ramo et al., 2013; see also **Chapter 1**) and this may have increased their role in seed dispersal, facilitating range expansions of plant species and genotypes. LBBG and white stork move at three spatial scales, enabling seed dispersal into a range of habitats: 1) daily movements of up to 20 km between different feeding and roost sites within the extensive ricefield complex, where they often stay for several days at a time (Bouten et al., 2013; see **Chapter 1**); 2) between ricefields and other habitats in Andalusia, including other agricultural lands and natural wetlands such as coastal marshes and inland shallow lakes, with direct flights concentrated within a radius of 100 km (Sanz-Aguilar et al., 2015, see **Chapter 1**); 3) long-distance migratory flights over hundreds of km to other parts of Europe or Africa. Between September to November, many gulls and storks are on passage to Africa (Baert et al., 2018; Flack et al., 2016). Gut retention times for seeds easily allow endozoochory over such distances (Green et al., 2016). However, movement patterns are changing in response to climate change and increases in food supply from anthropic habitats such as landfills (Tortosa et al., 2002), and these changes will also influence plant dispersal.

Conclusions and future work

Even though 92% of European angiosperms in continental Europe lack a fleshy fruit (Heleno & Vargas, 2015), avian endozoochory studies to date have concentrated on the remaining 8% (i.e. on frugivores). Our study illustrates the importance of avian endozoochory as a major means of dispersal for other angiosperms within and beyond a wetland landscape. Waterbirds provide maximum dispersal distances for many angiosperms that greatly exceed those predicted from their dispersal syndromes, with major implications for how plants respond to climate change, land use transformation or introductions of alien species.

Studying waterbird-plant dispersal interactions can improve our understanding of community structure, connectivity and distributions of plant species. The quality tracking data being generated on waterbirds provides an excellent opportunity for spatially explicit modelling of seed shadows in the future. Effective

dispersal also requires that seedlings become established in new habitats, and the potential for such establishment should be investigated. Detailed studies of *J. bufonius* are required to establish how endozoochory influences population genetics and phylogeography. More research is vital to address plant-bird dispersal networks involving larger numbers of waterbird species and families, and in natural habitats.

Acknowledgements

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Supplementary material

Jaccard Index equation for each combination of species and sample type:

$JI = a/(a + b + c)$, where

JI = Jaccard similarity coefficient,

a = number of plant species common to (shared by) gull/stork and faeces/pellets samples and transects,

b = number of species unique to the transects, and

c = number of species unique to the gull/stork and faeces/pellets samples

This index ranges from 0, when the two communities share no species, to 1 when there is complete overlap of species.

Table S1. List of plant taxa recorded in vegetation transects, and/or bird excreta samples. Twenty vegetation transects were carried out in two months (September and February), and the number in which each plant was recorded is listed.

Dispersal syndromes (dis.syn): endozoochory, epizoochory, barochory, hydrochory, myrmechory, anemochory or autochory (Julve, 1998); Ellenberg moisture values (Ellen) are from Julve 1998 and Hill et al., 1999; mean seed weight (mass in mg) is from Kleyer et al., 2008; presence in bird samples, and number of transects in which the species was recorded. Taxa listed in bold are alien species.

Family	Taxa	Dis. syn	Ellen	mass	Bird	N. of tran.
<i>Amaranthaceae</i>	<i>Beta maritima</i>	baro	4	-	-	4
<i>Amaranthaceae</i>	<i>Amaranthus albus</i> ^{a,b,c}	epi	5	0.35	LBBG, WS	3
<i>Amaranthaceae</i>	<i>Amaranthus retroflexus</i> ^{a,b,c}	epi	4	0.46	WS	-
<i>Araceae</i>	<i>Lemna</i> sp.	hydro	11	-	-	1
	<i>Lemna minor</i>	hydro	11	-	LBBG, WS	-
	<i>Lemna gibba</i>	hydro	11	-	WS	-
<i>Asteraceae</i>	<i>Conyza canadensis</i> ^{a,b,c}	anemo	4	0.05	-	10
<i>Asteraceae</i>	<i>Sonchus</i> sp.	-	-	-	-	7
<i>Asteraceae</i>	<i>Sonchus oleraceus</i> ^{b,c}	baro	5	0.33	-	7
<i>Asteraceae</i>	<i>Anthemis</i> cf. <i>Arvensis</i> ^{b,c}	baro	5	0.75	-	5
<i>Asteraceae</i>	Unidentified	-	-	-	-	2
<i>Asteraceae</i>	<i>Calendula arvensis</i>	epi	4	3.58	-	2
<i>Asteraceae</i>	<i>Helianthus annuus</i> ^{b,c}	baro	5	38.8	-	1
<i>Asteraceae</i>	<i>Senecio vulgaris</i> ^{b,c}	anemo	5	0.27	-	1
<i>Asteraceae</i>	<i>Sylbium marianum</i>	anemo	4	-	-	1
<i>Boraginaceae</i>	<i>Heliptropium europaeum</i>	baro	4	-	-	1
<i>Brassicaceae</i>	<i>Brassica napus</i>	baro	5	4.4	-	5
<i>Brassicaceae</i>	<i>Capsella bursa-pastoris</i> ^{b,c}	anemo	5	0.98	-	2
<i>Brassicaceae</i>	<i>Coronopus squamatus</i>	baro	5	1.87	-	2
<i>Brassicaceae</i>	<i>Barbarea vulgaris</i>	baro	6	0.6	-	1
<i>Brassicaceae</i>	<i>Diplotaxis erucoides</i> ^{b,c}	anemo	4	0.2	-	1
<i>Caryophyllaceae</i>	<i>Spergularia marina</i>	anemo	8	0.07	WS	1
<i>Caryophyllaceae</i>	<i>Stellaria mediab</i> ^c	baro	5	0.38	-	1
<i>Caryophyllaceae</i>	<i>Suaeda vera</i>	hydro	7	0.55	-	1
<i>Characeae</i>	<i>Chara</i> sp.	-	-	-	-	1
<i>Chenopodiaceae</i>	<i>Chenopodium</i> cf. <i>album</i>	-	-	-	-	3
<i>Convolvulaceae</i>	<i>Convolvulus arvensis</i> ^{b,c}	baro	4	11.9	-	1
<i>Cucurbitaceae</i>	<i>Ecballium elaterium</i>	auto	5	12.4	-	1
<i>Cyperaceae</i>	<i>Cyperus rotundus</i>	baro	7	0.24	-	1
<i>Cyperaceae</i>	<i>Cyperus difformis</i>	baro	10	0.03	LBBG, WS	-
<i>Cyperaceae</i>	Unidentified	-	-	-	-	6
<i>Elatinaceae</i>	<i>Bergia capensis</i>	auto	-	-	LBBG	-
<i>Euphorbiaceae</i>	<i>Chamaesyce serpens</i>	mirme	2	-	-	5
<i>Euphorbiaceae</i>	<i>Euphorbia pepus</i>	mirme	4	0.49	-	2
<i>Euphorbiaceae</i>	<i>Mercurialis ambigua</i>	mirme	4	-	-	1
<i>Fabaceae</i>	<i>Medicago polymorpha</i>	epi	-	2.9	-	7
<i>Fabaceae</i>	<i>Trifolium</i> sp.	-	-	-	-	5
<i>Fabaceae</i>	<i>Trifolium repens</i>	epi	5	0.59	WS	4
<i>Geraniaceae</i>	<i>Erodium</i> sp.	-	-	-	-	2
<i>Geraniaceae</i>	<i>Geranium molle</i>	auto	5	1.08	-	1
<i>Juncaceae</i>	<i>Juncus bufonius</i> ^{b,c}	epi	7	0.02	LBBG, WS	10
<i>Juncaceae</i>	<i>Juncus subnodulosus</i>	epi	7	0.09	LBBG, WS	1
<i>Malvaceae</i>	<i>Malva sylvestris</i>	baro	4	23.6	-	9
<i>Malvaceae</i>	<i>Malva</i> sp.	-	-	-	-	6
<i>Plantaginaceae</i>	<i>Plantago</i> sp.	-	-	-	-	1
<i>Poaceae</i>	<i>Polypogon monspeliensis</i> ^{b,c}	epi	8	0.13	WS	7
<i>Poaceae</i>	<i>Cynodon dactylon</i>	baro	6	0.14	-	5
<i>Poaceae</i>	<i>Poa annua</i> ^{b,c}	baro	6	0.22	-	4

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<i>Poaceae</i>	<i>Echinochloa crus-galli</i> ^{h,c}	epi	6	2.33	-	3
<i>Poaceae</i>	<i>Panicum milleaceum</i>	anemo	3	3.78	WS	-
<i>Poaceae</i>	<i>Sorghum cf. halepense</i>	epi	5	-	WS	-
<i>Polygonaceae</i>	<i>Polygonum aviculare</i>	baro	5	1.82	-	6
<i>Polygonaceae</i>	<i>Rumex sp.</i>	-	-	-	-	6
<i>Polygonaceae</i>	<i>Persicaria sp.</i>	-	-	-	-	3
<i>Portulacaceae</i>	<i>Portulaca oleracea</i> ^{h,c}	baro	2	0.33	LBBG, WS	2
<i>Ranunculaceae</i>	<i>Ranunculus sceleratus</i>	hydro	9	0.13	LBBG, WS	7
<i>Ranunculaceae</i>	<i>Batrachium sp.</i>	-	-	-	-	4
<i>Ranunculaceae</i>	<i>Ranunculus repens</i>	-	5	2.24	WS	2
<i>Solanaceae</i>	<i>Solanum nigrum</i> ^{h,c}	endo	5	0.91	LBBG	4
<i>Solanaceae</i>	<i>Solanum dulcamara</i>	endo	8	1.59	LBBG, WS	-

Table S2. Summary test of the effect of species, sample type, period and diet in seed composition (for all taxa other than *Juncus bufonius*) based on a multivariate generalized linear model (manyglm in the *mvabund* package in R) with a negative binomial distribution. Test statistics (Z) were calculated assuming no collinearity between response variables. P-values (p) were determined using 1000 resampling iterations, using probability integral transform (PIT) residuals. Significant p-values are shown in bold.

	Species		Period		Sample type		Diet	
	Z	p	Z	p	Z	p	Z	p
<i>Juncus subnodulosus</i>	5.729	0.118	7.598	0.347	0.569	0.999	0.741	0.999
<i>Sorghum halepense</i>	1.861	0.943	1.848	0.994	2.446	0.853	5.894	0.454
<i>Panicum milleaneum</i>	1.856	0.971	1.835	0.994	2.395	0.897	4.786	0.674
<i>Amaranthus albus</i>	0.448	0.976	3.821	0.944	0.866	0.998	0.913	1
<i>Amaranthus retroflexus</i>	1.856	0.971	1.835	0.994	2.395	0.897	0.399	1
<i>Chara sp.</i>	2.853	0.746	3.326	0.944	0.273	1	1.27	1
<i>Polygonum monspeliensis</i>	3.712	0.569	5.98	0.672	1.246	0.998	3.047	0.965
<i>Trifolium repens</i>	1.856	0.971	1.835	0.994	2.395	0.897	4.786	0.674
<i>Solanum nigra</i>	3.037	0.673	7.796	0.352	0.096	1	2.735	0.979
<i>Solanum dulcamara</i>	0.987	0.976	7.311	0.389	0.03	1	0.837	1
<i>Ranunculus sceleratus</i>	6.652	0.086	17.13	0.004	1.195	0.998	2.017	0.994
<i>Ranunculus repens</i>	1.856	0.943	1.835	0.994	2.395	0.883	0.399	1
<i>Chenopodium cf. album</i>	3.73	0.51	3.716	0.944	0.007	1	0.934	1
<i>Poaceae</i>	0.09	0.976	2.263	0.994	0.242	1	2.178	0.994
<i>Cyperus difformis</i>	6.102	0.096	12.79	0.033	0.247	1	0.044	1
<i>Lemna minor</i>	0.443	0.976	3.482	0.944	0.562	1	1.29	1
<i>Lemna gibba</i>	3.712	0.569	5.98	0.672	0.483	1	4.217	0.818
<i>Spergularia sp.</i>	1.856	0.943	2.277	0.994	0	1	0.063	1
<i>Portulaca oleracea</i>	1.306	0.971	5.14	0.821	0.545	1	1.167	1
<i>Bergia capensis</i>	1.006	0.976	2.742	0.981	1.096	0.998	1.336	1

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Spatial patterns of weed dispersal by wintering gulls within and beyond an agricultural landscape



Martín-Vélez V., van Leeuwen C. H. A., Sánchez M.I., Hortas F., Shamoun-Baranes J., Thaxter C. B., Lens L., Camphysen C. J. & Green A. J. Spatial patterns of weed dispersal by wintering gulls within and beyond an agricultural landscape. Submitted to *Journal of Ecology*.

Abstract

Non-frugivorous waterbirds disperse a broad variety of vascular plants by endozoochory, providing longer dispersal distances than other mechanisms. Many waterbirds exploit agricultural landscapes (e.g. ricefields), potentially functioning as vectors of weeds within and between agricultural landscapes. The expansion of gulls inland to exploit anthropic habitats worldwide has important implications for the spread of weed species and genes (e.g. for herbicide resistance). Yet, to date there are no studies on the spatial distribution of weed dispersal by waterbirds.

We developed a plant dispersal model based on movements of 19 lesser black-backed gulls *Larus fuscus* using ricefields, via GPS telemetry. We combined daily movements of gulls with two curves estimating the retention times (RT) of seeds in their guts: 1) an experimental curve based on RT in captivity for four weeds known to be dispersed by gulls: *Juncus bufonius*, *Cyperus difformis*, *Polypogon monspeliensis* and *Amaranthus retroflexus*; 2) a theoretical curve based on the scaling relationship between body mass and mean RT.

The theoretical RT model showed higher median dispersal distances than the experimental RT model (960 m versus 690 m) but lower maxima (131 versus 151 km), yet spatial patterns of weed deposition were similar and depended on gull movements. There was little change in the frequency distribution of seed dispersal distances, when the most mobile individuals were removed from the model. This suggests that variation between individual gulls had little influence on seed shadows. About 92% of seeds were dispersed within the ricefield area of 370 km², representing >10,000 seeds a day, most of which were moved between different individual fields. The remaining 8% of seeds were deposited beyond ricefields into other habitats, 42% of which reached moist environments (other irrigated agriculture, rivers and natural wetlands) particularly suitable for weed establishment.

This is the most detailed spatial study of weed dispersal by waterbirds, and reaffirms the importance of their overlooked role as vectors of plants widely

assumed to disperse over much shorter distances by abiotic means. Attention should be paid to non-frugivorous birds in order to predict and manage expansion of weeds and alien plants.

Introduction

Seed dispersal plays a central role in the meta-population dynamics and long-term persistence of plant species (Willson & Traveset, 2000). Plant seed dispersal is of particular interest for weeds (i.e. plants that spontaneously grow on land modified by humans, Bourgeois et al., 2019), because many weeds cause high economic cost to agriculture and many are invasive species with severe ecological and societal impact. For example, estimated costs and yield loss due to weed management in Australia exceeded \$ 1,000 million in 2002 (Sinden et al., 2004). Nevertheless, we know little about the role of birds that use anthropogenic (e.g. agricultural) habitats in weed dispersal.

Dispersal via gut passage (endozoochory) is ideal for long-distance seed dispersal, but it has been widely, yet erroneously, assumed that this mechanism is limited to frugivorous animals (i.e. those feeding on fleshy fruits) (Costea et al., 2019). Only plants with a fleshy fruit are assigned to an “endozoochory syndrome” used to predict dispersal mechanisms, and only 8% of the European flora and 1.5% of agricultural weeds worldwide have such a syndrome, compared to 63% and 80% respectively for an unspecialized syndrome or “barochory” (Benvenuti, 2007; Heleno & Vargas, 2015). Therefore, syndromes imply that animals are of little importance for the dispersal of weeds. On the other hand, empirical data demonstrate that seeds dispersed by animals tend to be dispersed further than seeds dispersed by abiotic mechanisms, including wind (Vittoz & Engler 2007; Bullock et al., 2017). Hence, weed dispersal by animals may have relatively great impact.

Recent studies have demonstrated that granivorous and omnivorous waterbirds such as ducks or gulls disperse a high diversity and abundance of seeds from angiosperms with no fleshy fruit (NFF from hereon) by endozoochory, including many agricultural weeds (Soons, et al., 2016; Farmer, et al., 2017; Lovas-Kiss et

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al., 2018ab). NFF seeds dispersed by waterbird endozoochory seem to be just as adapted to survive gut passage as those from fleshy-fruited plants (Costea et al., 2019). Fixation on morphological syndromes leads to the systematic overlooking of endozoochory for weeds by non-frugivorous birds likely disperse seeds over great distances. Especially in human-modified landscapes, we can expect that endozoochory of weeds by waterbirds will greatly increase the probability of seeds dispersing out of a given agricultural field, beyond adjacent fields, and into other habitats including other crops. Understanding and controlling the spread of weeds between fields is particularly important, because even a single herbicide-resistant plant colonizing a field can lead to total field infestation (e.g. Palmer Amaranth *Amaranthus palmeri* took over cotton fields within three years; Norsworthy et al., 2014).

Spatial modelling of plant dispersal is a major research tool in plant ecology because of its importance in predicting plant distribution and population dynamics (Husband & Barrett 1996; Jeltsch et al., 2008), as well as for implementing effective management strategies (Aben et al., 2016). In recent decades, data have been collected on the retention time of seeds in the avian digestive system, both for frugivores and waterbirds (Lovas-Kiss et al., 2020), in order to estimate potential dispersal distances. Experimental retention time data have been combined with animal movement data in mechanistic models that estimate seed dispersal distances. However, most models to date focused on dispersal by frugivorous birds (Côtés & Uriarte, 2013; Godínez-Alvarez et al., 2020), and no studies have applied this approach to questions about dispersal of weeds by non-frugivorous birds.

Methodologically, most previous studies of waterbird endozoochory have estimated dispersal distances simply by multiplying mean retention times by the average flight speed, bypassing a need for empirical movement data (Farmer et al., 2017; Raulings et al., 2011; Nogales et al., 2001). This likely overestimates seed dispersal distances, and new technology using GPS tracking devices can reduce uncertainty of bird movements involved in seed dispersal compared to radio-tracking studies (e.g. Bartel et al., 2018). High-resolution data allow the creation of more reliable mechanistic models in which the speed, direction and

duration of bird movements are not fixed parameters, and individual variation in movement patterns can be taken into account.

Understanding the role that waterbirds play in the dispersal and spatial dynamics of weeds will help to predict their spread and to develop new management strategies in agricultural landscapes. Here, we aimed to assess the importance of non-frugivorous bird movements for the dispersal of weeds throughout a human-modified landscape. We present a case study centred on Doñana ricefields, Spain's largest rice-growing area many waterbirds (e.g. gulls) rely on such ricefields to feed during harvesting. The lesser black-backed gull (LBBG) *Larus fuscus* has been increasing in the ricefields (Rendón et al., 2008) and it is known to disperse a wide range of plants while feeding there (Lovas-Kiss et al., 2018a). Furthermore, recent movement studies showed the importance of the ricefields for LBBG during the non-breeding season (vanRees et al. 2020; **Chapter 1**). Using GPS data on LBBG movements, we model their potential to disperse NFF weeds between different rice fields, as well as beyond the ricefields into other anthropic and natural habitats. The weeds considered in this study impact ricefields as well as other crops in the same region (e.g. cotton, wheat, vineyards).

The specific objectives of this study are: 1) model weed deposition based on two different retention curves: one from an experiment with captive gulls, and another based on the scaling relationship across bird species between body mass and gut retention times; 2) determine the extent to which gulls disperse weeds over distances that exceed field boundaries by quantifying seed dispersal distances, spatial seed shadows and the habitat types into which seeds are deposited; 3) determine the intra-specific variation among gulls in their contribution to seed dispersal.

Materials and methods

Study area and species

The ricefields (37,000 ha) in the Guadalquivir marshes in Andalusia account for up to 42% of the total rice production in Spain. They are located in the surroundings of Doñana National Park and World Heritage Site, and are part of one of the most important wetland complexes for waterbirds in the Western Palearctic (Rendón et al., 2008; Green et al., 2018). These ricefields provide important habitat for many wintering waterbirds that use them both for feeding and roosting, especially during and just after the rice harvest (Rendón et al., 2008; Toral & Figuerola, 2010).

The lesser black-backed gull *Larus fuscus* (LBBG) is migratory, breeds in Northern Europe (Baert et al., 2018) and winters in Southern Europe. Due to its opportunistic and generalist diet, the LBBG has largely switched from marine to inland habitats to exploit resources of anthropogenic origin such as ricefields and landfills (Ramos et al., 2009), and is now the second most numerous wintering waterbird in Andalusian wetlands, with a population of over 70,000 (**Chapter 1**). Up to 15,000 gulls are regularly counted in the ricefields west of the Guadalquivir river (the only ones covered in aerial counts), with peaks in early winter during the harvest (Rendón et al., 2008). The ricefields are the most important node in the network of sites used by LBBG in Andalusia (**Chapter 1**). This network shows high functional connectivity between different habitat types (**Chapter 1**), increasing the potential of LBBG as vectors for seeds, including those of agricultural weeds. During the harvest, LBBG feed mainly on alien red swamp crayfish *Procambarus clarkii* exposed as the rice is harvested, and they disperse seeds carried on the outside of the crayfish, or ingested while feeding on rice grains (Lovas-Kiss et al., 2018a). Gulls concentrate their feeding during the day on fields where harvesting or disking is underway, later moving to dykes and other roosting sites.

Gull tracking data

A large number of gulls have been equipped with Global Positioning System (GPS) trackers, as part of the University of Amsterdam Bird Tracking System (UvA-BiTS) (Thaxter et al., 2015; Shamoun-Baranes et al., 2017, Baert et al., 2018), stored in a centralized database (<http://www.uva-bits.nl>; Bouten et al., 2013). For this study, we first extracted all data points within the rectangle created between latitudes of 36.0°- 40.5° N and longitudes of 0.0°-9.0° W (i.e. the whole of Andalusia) between September and December from 2010 to 2017. These months cover the harvesting period when gulls feed within the ricefields, although fields often remain flooded for some weeks in January, when they may be used as roost sites for gulls feeding in landfills (VanRees et al., 2020; **Chapter 1**).

For our analysis, we used the following parameters recorded by the GPS loggers: body mass, date, time of the day (UTC), latitude, longitude and instantaneous speed. Position accuracy for a stationary logger transmitting a signal every 10 min was on average 30 m [range 9-108 m] (Bouten et al., 2013). We calculated additional variables from the raw parameters: Harversine distance between fixes, time difference between GPS points (calculated from forward intervals between consecutive GPS points set up between 10-60 min) and trajectory speed (km/h as distance in km divided by time difference in h). We filtered the dataset for quality by deleting any fix with a trajectory speed greater than 80 km/h, which is the realistic limit for bird flight speeds (Klaassen et al., 2012). We also deleted gaps in the trajectories of greater than 120 minutes and assumed they were due to low battery power.

We selected all GPS points within the Doñana ricefields based on the CORINE Land Cover (CLC) 2012 habitat type (EEA, 2012). Based on the speed histogram within the ricefields, we classified the data as either “stationary” or “active flight”, taking 13 km/h (i.e. 3.6 m/s) as the threshold (Fig. S2). We assumed “active” represented displacements of gulls between foraging or roosting sites that are relevant for seed dispersal, and that no seed ingestion occurred during the night because gulls are diurnal foragers. We further assumed that seed ingestion

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occurred within ricefields between 0700 and 1700 h UTC according to the timing of “active flying” (Fig. S3). This time range excluded the main roosting periods (especially night time) when seed ingestion is improbable (see also **Chapter 4** for diel rhythms of LBBGs). The GPS points within the ricefields correspond to a total of 1,867 gull-days, from a combination of 8 different winters from 2010 to 2017 and 19 different individual gulls from five different breeding colonies (eight individuals from Zeebrugge, Belgium; five from Texel, the Netherlands; three from Skokholm and three from Walney, UK). Up to nine individuals were present in a given winter, and each individual was present for between one and four winters.

In order to calculate the probability distribution of seed dispersal events according to dispersal distance (i.e. the “dispersal kernel”, Nathan et al., 2012), data on retention times of seeds in the digestive system are required. To estimate the time elapsed between seed ingestion and excretion, we used both experimental and theoretical approaches. Feeding experiments using captive waterbirds have provided valuable information on factors influencing gut retention times, such as seed size and hardness, or diet (e.g. Charalambidou et al., 2005; van Leeuwen et al., 2012a; Lovas-Kiss et al., 2020), but it is unclear to what extent results from captivity (where birds are in cages) represent retention times under natural conditions (with actively moving birds), given the influence of activity on digestion (van Leeuwen et al., 2012b). We therefore assessed retention times experimentally, but compared our results with a theoretical retention time curve based on body mass.

Experimental seed retention time

A seed retention curve was calculated from an experiment carried out in January 2020 with seven captive LBBG individuals as part of a related study (see Ansoategui et al. submitted for detailed analysis of individual seed species and germination). Individuals were captured during January 2020 in a waste management plant near Seville, Spain. For this study, we used data for four weed species lacking a fleshy fruit (200 seeds each per gull of toad rush *Juncus bufonius*, small-flowered nut sedge *Cyperus difformis*, annual beard grass *Polypogon monspeliensis* and

common amaranth *Amaranthus retroflexus*; a combined total of 5,600 seeds for all gull individuals). These weeds are associated with rice crops and are four of 15 angiosperm species known to be dispersed by LBBG in the study area (Table S1). *C. difformis* is strongly associated with rice and is considered one of the world's 40 worst weeds (Bryson & Carter, 2008). The other weeds inhabit a broad range of disturbed habitats and crops (Agroatlas, 2020), e.g. *J. bufonius* is a weed of wheat and barley (Broster et al., 2012). Herbicide resistance has been recorded for all but *J. bufonius* (Heap, 2020). *J. bufonius* and *A. retroflexus* are considered noxious weeds by Bourgeois et al. (2019).

To facilitate feeding, the seeds were mixed with a bread pellet of about 2 cm diameter and fed to the gulls. Gulls were fed with sardines ad libitum for the duration of the experiment. After seed ingestion, faecal samples were collected in time intervals: first after half an hour, then every hour until the first six hours, then every two hours from 6 to 12 h, and then every four hours until 34 h (the end of the experiment). In the laboratory, faeces were sieved through a 40 μm mesh and intact seeds were separated, counted and placed in climatic chambers for germination. To generate the retention time curve (Fig. 1A), we assumed the retention time of any recovered seed was the midpoint between collection times (e.g. if a seed came from faeces collected at 2.5 h and the previous collection was at 1.5 h, we assigned a retention time of 2h). Seed retention times have been overestimated during previous captive studies by assuming they correspond to the timing of faecal collection (i.e. 2.5 h in the above case).

Theoretical seed retention time

To test objective 1, we tested the robustness of our final results and the importance of the retention time distribution in the seed dispersal model using a theoretical retention time curve (Fig. 1B). We estimated a curve based on an allometric equation relating mean retention time to body mass in birds (Yoshikawa et al., 2019). Using a mean body mass of 845 ± 127 SD g (from the 19 gulls we tracked), this equation gives a mean retention time of 3.1 h. Using this mean value, we fitted a lognormal curve distribution, which best predicts seed retention times (Viana et al., 2016b).

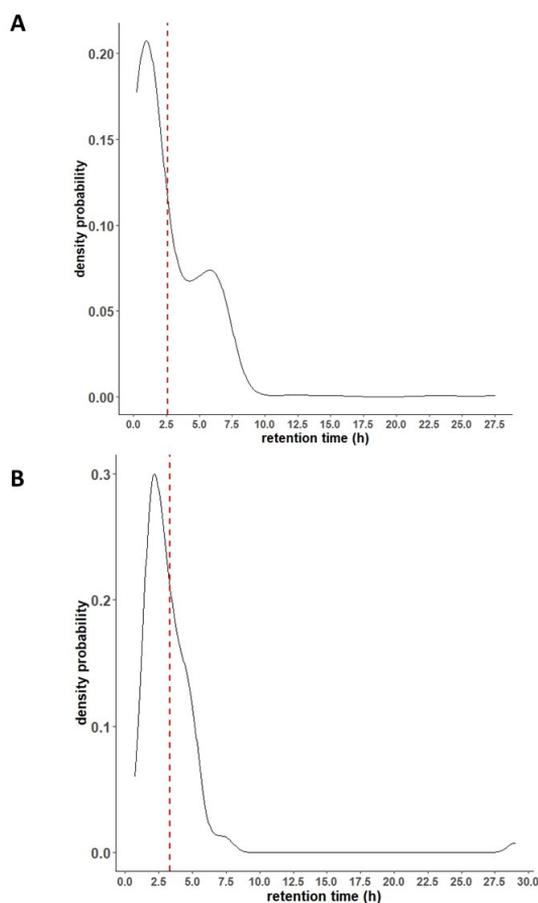


Figure 1. A) Experimental retention curve (density probability on y axis and retention time in hours on x axis, bandwidth=1) generated from 4465 seeds recovered from four plant taxa used in a captive experiment. B) Theoretical curve derived from random sampling (N=200) based on 3.1 h mean retention time (calculated from Yoshikawa et al., 2019) and a lognormal curve (after Viana et al., 2016b). Red dashed lines indicate mean retention times.

Seed deposition modelling

To test objective 2, we randomly selected one of the 1,867 gull-days and assumed that a seed was ingested at a time t_0 randomly assigned to a point between 0700 h and 1700 h. Based on the experimental or the theoretical retention time curve,

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we randomly selected a retention time t_1 when the seed would be egested, based on the density probability from the curve through the *sample* function in R (v3.6.3 R Core Team, 2020). The location of seed egestion was calculated based on the GPS point at t_1 , and the distance the seed was dispersed was calculated based on the Euclidean distance between GPS points at t_0 and t_1 . We repeated such randomizations 10,000 times for each of the experimental and theoretical models. Thus, each simulation represented the dispersal of 10,000 seeds in the study area. The mean number of intact seeds per LBBG faecal sample in the field is approximately 1 (**Chapter 2**; Lovas-Kiss et al., 2018a), and each sample represents around 12.5% of daily faecal output (Hahn et al., 2007). Therefore, each simulation represented less than the number of seed dispersal events by LBBG in a typical day, as the number of LBBGs in the ricefields exceeds 15,000 (Rendón et al., 2008).

To test objective 3 for model sensitivity to individual variation in trajectory movements, we ran the model again after removing the two of the 19 individuals with the longest geometric mean seed dispersal distances, according to model outputs. We then removed sequentially the four, six and eight individuals with the longest mean dispersal distances. This enabled us to assess the influence of individual variation on the cumulative frequency distributions of the dispersal distance curves.

We summarized dispersal distances using geometric means instead of arithmetic means because their distribution was strongly right-skewed. However, these geometric means were consistently lower than the medians (Table 1). For spatial model visualization, we first projected the seed point coordinates to UTM and applied the *Point Density* tool in Arc Map 10.4. We applied a cell size of 100 m and a search radius for neighbouring cells of 1 km. We set up the output values based on standard deviations as default to visualize the densities measured as number of seeds km^{-2} . Furthermore, we overlapped the seeds deposited outside of the ricefields with a regional land use shapefile (Junta de Andalucía, 2013) with more detailed land uses than Corine Land Cover. This allowed us to determine the main habitat types into which seeds were dispersed. The six main habitat categories were: river, other water bodies (e.g. lakes or reservoirs),

natural vegetation, urban, permanent irrigated agriculture and other agriculture (details in Table S3). As three of the weeds used for the study require moist soils (with high Ellenberg values, Table S1), habitats closely associated with water were considered suitable for weed deposition (e.g. other irrigated agriculture, river, waterbodies; Table S3).

Results

Experimental seed retention times

There were no significant differences in the retention times among seed species (Kruskal-Wallis chi-squared = 2.77, $df = 3$, $p = 0.429$). We therefore summed all seed species to generate an overall retention time curve, based on a total of 4,465 intact seeds recovered from the seven individuals (Fig. 1A). Mean retention time was 2.6 h and the last seeds were recovered after 29 hours. From 16 to 32% of seeds germinated per species (details in Ansotegui et al., submitted).

Model based on experimental retention times

The geometric mean dispersal distance of 10,000 seeds from randomisations based on experimental retention times was 0.58 km (Table 1). More than 7% (723) of the seeds were dispersed outside of the ricefields (see white dots in Fig. 2). Geometric mean dispersal distance for seeds egested within ricefields was 0.45 km with a maximum of 36.7 km, compared to a geometric mean of 14.3 km and maximum of 151 km for seeds egested outside of ricefields (Table 1).

There were statistically significant differences in the dispersal distances recorded between the 19 gull individuals (Kruskal-Wallis chi-squared = 283.98, $df = 18$, $p < 0.001$). However, sensitivity analyses showed no consistent reductions in mean seed dispersal distances when those pairs of gull individuals with longest geometric mean dispersal distances were removed sequentially from the model (Table S2). Of the 19 individuals, three had a maximum seed dispersal distance exceeding 100 km.

Table 1. Comparison between models with experimental and theoretical retention time curves, presenting numbers of deposited seeds and dispersal distances (in km, geometric means, quantiles and maximum distances). Results are given for all seeds combined (total), seeds deposited within ricefields (percentage in brackets) and seeds deposited outside of ricefields. Note 50% quantiles are the medians, and arithmetic means would be considerably higher (e.g. 3.51 km for Experimental total and 4.05 km for Theoretical total).

Model		N° deposited seeds	Dispersal distance (Km)				
			Geometric mean (95% CI)	25%	50%	75%	Max
Experiment	total	10,000	0.58 (0.56-0.60)	0.16	0.69	2.33	150.96
	within	9,277 (92.77%)	0.45 (0.43-0.47)	0.14	0.57	1.81	36.96
	outside	723 (7.2%)	14.3 (12.95-15.80)	8.25	17.88	41.82	150.96
Theoretical	total	10,000	0.9 (0.87-0.93)	0.27	0.96	2.74	130.68
	within	9,174 (90.7%)	0.7 (0.68-0.72)	0.24	0.8	2.11	40.65
	outside	826 (8.26%)	14.75 (13.46-16.16)	9.42	17.97	42.57	130.68

Areas with the highest densities of deposited seeds (exceeding 194 seeds km⁻²) were restricted to the ricefield area and to the river that divided it (Fig. 2). Seeds egested outside the ricefield landscape were deposited mainly in urban habitats (42.2%) that are less likely to be suitable for these weed species, including three solid waste treatment plants/landfills (24.9%) (Fig. 2 and 3). However, 34.8% of the seeds were deposited in agricultural lands, including permanently irrigated lands (25.3%), and other agricultural lands (9.5%) such as olive groves and vineyards (details in Table S3). Some seeds (9.4%) were dispersed within the stretch of the Guadalquivir river (Fig. 3) that runs through the middle of the ricefield area (Fig. 2). An important fraction of seeds (8%) were deposited in other waterbodies (Fig. 3), including protected areas such as the nearby Doñana Natural Space, and Fuente de Piedra Lake situated at 118 km from the nearest ricefield (Fig. 2). Finally, 5.5% of the seeds were deposited in areas of natural vegetation such as grasslands or oak woodlands (Fig. 3). Around 42% of the seeds egested outside the ricefields were deposited in an environment with high water availability that is most likely to enable weeds to establish.

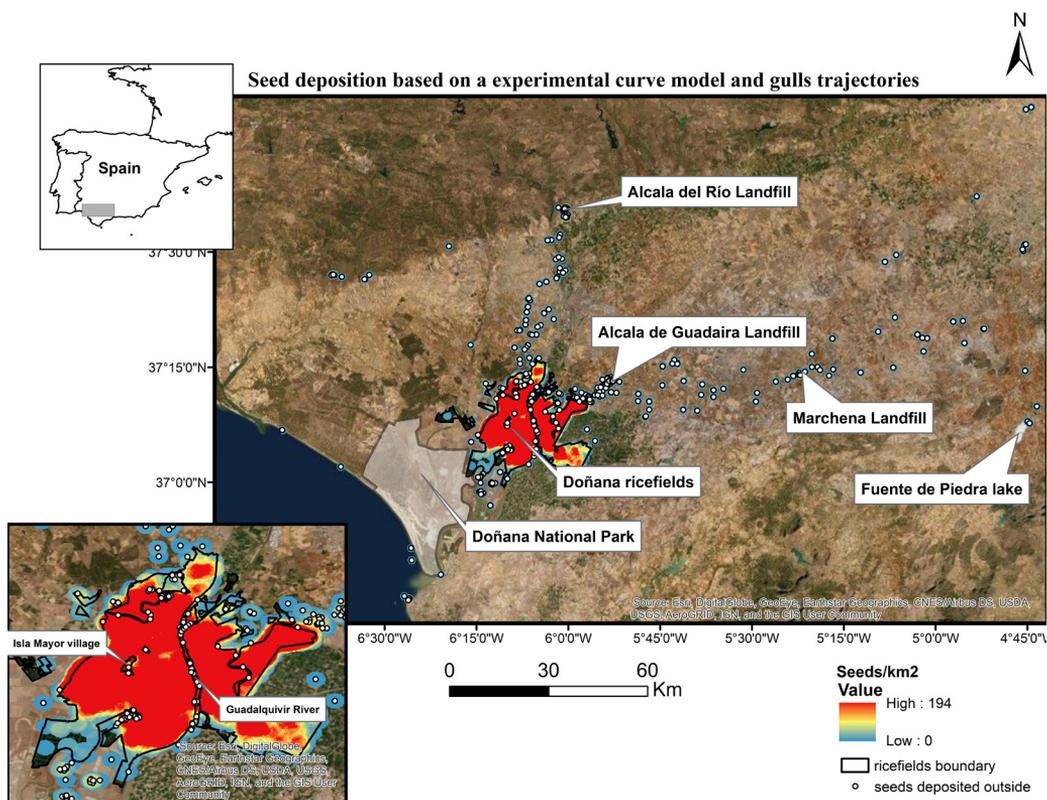


Figure 2. Point density distribution of 10,000 seeds when dispersal was modelled using an experimental retention time curve and GPS trajectories of 19 gulls. Red areas show sites where the highest densities of seeds (maximum 194 km⁻² are deposited), which coincides with the ricefield area (close up in the bottom left hand corner). Blue areas indicate lower density of seed deposition. Each white dot represents one seed deposited outside ricefields. Seeds deposited in ricefields (92% of the total) are not shown individually.

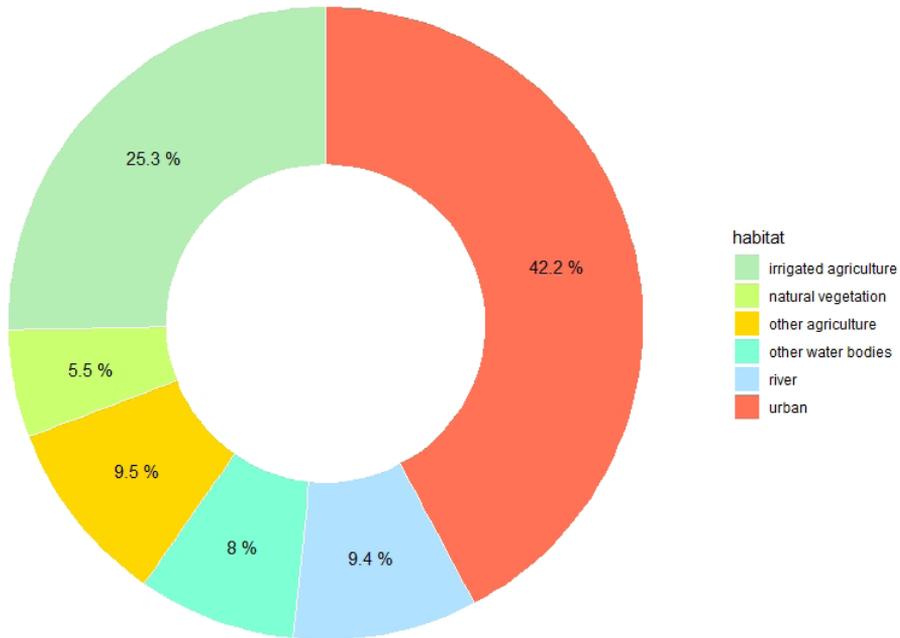


Figure 3. Frequencies of seed dispersal into habitat types outside ricefields, according to simulations using experimental retention times, based on Andalusian Government land use classification. See Table S3 for more detailed classification.

Model based on theoretical retention times

We found a difference between estimated dispersal distances based on experimental and theoretical retention time curves. Geometric mean dispersal distance from the model based on a theoretical retention time curve was 0.9 km, 55% more than for the experimental model (Table 1). This shift is in line with the greater mean retention time in the theoretical curve (Fig. 1). The theoretical model predicted significantly longer dispersal distances than the experimental model (Wilcoxon Test = 44804140, $p < 0.0001$). Cumulative frequency distributions showed that the seed dispersal distances predicted by the two models diverged considerably for distances of < 1 km, but then converged (Fig 4A, see also Table 1).

According to the theoretical model, over 8% (826) of seeds were deposited outside of the ricefields (Table 1). Dispersal distances for seeds moved outside the ricefields were only slightly higher in general than for the experimental model (Table 1, Fig. 4B), and the maximum dispersal distance (131 km) was lower. This was consistent with the retention time curves, as more seeds were recovered experimentally after ≥ 5 h than expected according to the theoretical curve (Fig. 1). Areas within the ricefields with the highest densities of >186 seeds km^{-2} , and spatial seed deposition patterns were similar to those found using experimental retention times (Fig. S1).

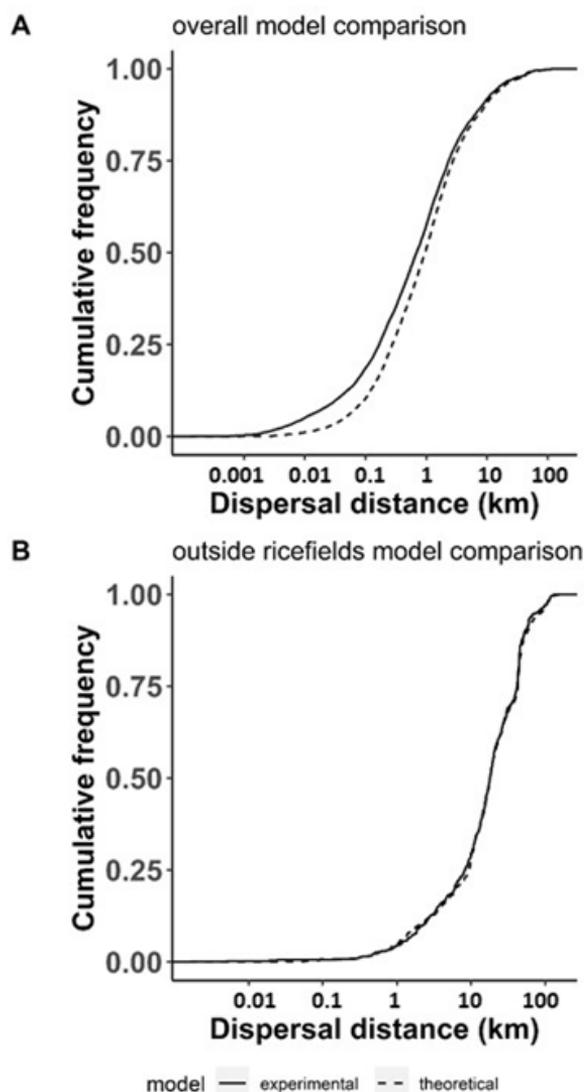


Figure 4. Cumulative frequency distributions of dispersal distances (km) obtained from models using experimental (solid line) and theoretical (dashed line) retention times, with a log scale on the X axis. A) based on all 10,000 seeds modelled. B) only for seeds deposited outside ricefields (723 for experimental and 826 for theoretical models).

Discussion

We compared experimental and theoretical models with different retention time curves, and found significant differences in seed dispersal distances for LBBG (our objective 1). We quantified seed shadows (objective 2), and found that several thousand weed seeds are dispersed over distances exceeding 2 km on a daily basis, for just one of the waterbird species frequenting the ricefields. LBBG often disperse weeds from ricefields into other suitable habitats. Variation in movement between individual gulls did not determine the final model output (objective 3).

All plant species used in this study were NFF weeds previously recorded in LBBG excreta from the ricefields (Lovas-Kiss et al., 2018a; **Chapter 2**). The probability of weed establishment depends on the suitability of the microhabitat where the seed is deposited, and germinability. Around 20% of seeds from LBBG excreta collected in ricefields later germinated in the lab, although this was reduced by the delay between sample collection and germination tests (Lovas-kiss et al., 2018a; **Chapter 2**). The number of seeds dispersed in our models is less than the number expected during a single day in the ricefields, given the numbers of gulls present and their rates of seed egestion (see Methods).

Ours represents the most complete spatial study of seed dispersal by waterbirds to date, being the first to combine high-resolution movement data in the landscape with local information on what plant taxa are actually dispersed by endozoochory. Kleyheeg et al., (2017) modelled seed dispersal by mallards using similar high resolution GPS data, and reported seed dispersal distances of 0.6-3 km. However, they did not have empirical data on the plant taxa dispersed in their study area, nor specific retention time data for those taxa. We found a high frequency of seed dispersal distances of >3 km in LBBG because they regularly shifted their roosting places in a dynamic response to the rice harvest cycle (**Chapter 1**; van Rees et al., 2020), whereas wintering mallards often remain within a limited home range for many weeks at a time (Sauter et al., 2012; Kleyheeg et al., 2017).

Our results based on GPS data confirm that previous studies assuming a straight, continuous flight (e.g. Nogales et al., 2001, Farmer et al., 2017) overestimate the seed dispersal distances for waterbird vectors. Based on the maximum retention time from the experimental curve (27.5 h) and an average flight speed of about 40 km/h (Klaassen et al., 2012), seeds would be expected to cover far greater distances of up to 1,000 km. This is unrealistic, partly because birds generally roost at night, even during seasonal migrations between breeding and wintering grounds, when LBBG make many stopovers (Klaassen et al., 2012, Shamoun-Baranes et al., 2017).

Importance of avian vectors for weeds

The dispersal distances obtained in our results underline the importance of avian endozoochory for NFF plants when compared to the dispersal distances expected for abiotic mechanisms, including wind. Tamme et al. (2014) suggested that *J. bufonius* had a maximum dispersal distance (MDD) via wind of only 100 meters, which is exceeded by over 75% of seeds dispersed by LBBG (Table 1). *Juncus bufonius* is the dominant seed in LBBG excreta from ricefields (Lovas-Kiss et al., 2018a; **Chapter 2**), and we found MDDs over 100 km, i.e. $>10^3$ greater than the MDD expected by wind. Similarly, Dauer et al., (2006) estimated the wind dispersal distances of the horseweed *Conyza canadiensis* (present in our study area, **Chapter 2**) as between 30-100 m. *Cyperus difformis* has been assigned to a barochory syndrome, and our other study weeds to an epizoochory syndrome (Table S1; Julve, 1998). Epizoochory is unlikely to provide dispersal distances that approach those provided by LBBG, as rodents are the only mammals abundant in ricefields, and the main vectors for epizoochory are the crayfish predated by the gulls and other birds (Lovas-Kiss et al., 2018a). Human vectors are hugely important for long-distance dispersal of weeds, especially between fields managed by the same people or otherwise strongly connected by human movements (Benvenuti, 2007). However, the seed shadows generated by avian and human vectors, and the habitats they are dispersed into, are likely to differ greatly.

Seed dispersal distances for gull endozoochory also generally exceed those recorded for frugivorous birds, which are mainly <200m with maxima of 1.5 to 14.5 km in most cases, although these are still longer dispersal distances than from abiotic mechanisms (Wenny et al., 2016; Bullock et al., 2017). Other waterbirds, such as storks or egrets, feed on crayfish and rice in the ricefields in a similar manner to LBBGs, and are certain to increase rates of weed dispersal. White storks and LBBG alone are expected to disperse in the order of 105 seeds per day (**Chapter 2**). However, seed shadows may differ for each waterbird species as they have different movement patterns. The weeds included in our models are also likely to be dispersed by wintering ducks that feed in the ricefields at night and then roost by day in other wetlands (Farmer et al., 2017; Marty et al., 2020). Other granivorous birds such as corvids and game birds are likely to be key vectors of NFF weeds via endozoochory in other ecosystems (Orlowski et al., 2016; Green et al., 2019), and are also worthy of future research and spatial modelling.

Influence of different retention time curves, and individual variation in movement

Individual variation in behaviour and gut functioning can have important consequences for seed dispersal (Zwolak, 2018). We tested the influence of individual movement behaviour, and the removal of the individuals showing the longest dispersal events did not change the shape of the dispersal kernel or the average dispersal distances (Table S2). The seed shadows generated from our models did not depend strongly on particular individuals with high mobility, but were the consequence of daily movements of all individuals.

Experimental studies of waterbird endozoochory have revealed strong variation in gut retention times and NFF seed survival between individual birds (Figuerola et al., 2010; Kleyheeg et al., 2015), and retention time curves are influenced by many factors including diet, age, stress and gender (van Leeuwen et al., 2012b; Kleyheeg et al., 2018). We did not consider the differences between seven individual gulls used to generate our experimental retention time curve, but instead we compared it with a theoretical curve generated from a meta-analysis.

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Our experimental retention curve showed shorter dispersal distances (median 690 m) than the theoretical retention curve (median 960 m), but both had a long tail typical of seed dispersal curves (Viana et al., 2013; Morales & Carlo, 2006; Anderson et al., 2011). As would be expected (Godínez-Álvarez et al., 2020), the higher mean retention time for the theoretical model generated longer dispersal distances for most seeds. However, the seed shadows were generally similar for the two models, especially for dispersal outside ricefields. Within ricefields, the theoretical curve led to a stronger increase in dispersal distances, associated with an increased probability that a gull had moved to a nocturnal roost site before seed egestion occurred. Median distances for seed dispersal by LBBG are likely to be lower in ricefields than in some other habitats, since LBBG have been found to move distances of 40 km to 80 km on a daily basis to reach feeding sites in previous studies (Thaxter et al., 2015, **Chapter 4**).

It is unclear whether the most accurate retention time curves are generated by experiments in captivity or by theoretical curves (Viana et al., 2013, Yoshikawa et al., 2019). Results in captivity may be influenced by the lack of flying activity. Kleyheeg et al., (2015) found that seed passage through the digestive tract of mallards increased by up to 80% with physical activity compared with animals resting in conventional cages, but there was little effect on the shape of the retention time curve. We did not study long-distance migratory flights, hence captive experiments may reasonably reflect the field conditions in which gulls were spending the majority of the day stationary, and often roosting after completing a feeding bout.

Normally, variation in traits such as seed size and hardness influence the retention time curves of individual NFF taxa (Figuerola et al., 2010; Lovas-Kiss et al., 2020). However, in our case all taxa showed the same retention curves, so the seed dispersal kernels generated from our models were equally valid for the four different taxa. Similar seed shadows can be expected for other plants dispersed by LBBG in our study area (Table S1).

Seed dispersal within the ricefield complex

A strong majority (around 92%) of dispersed seeds were deposited within the ricefield area, in both models. This was expected given the dominance of gull-days in which birds remained within the 360 km² ricefields, which provide both foraging and roosting habitat, and are so extensive that seeds can be dispersed up to 41 km and still be deposited within ricefields (Table 1). The spatial dynamics of the rice harvest, in which different fields are harvested sequentially rather than simultaneously, provides enough resources for individual gulls to remain in the area for days or weeks at a time (**Chapter 1**, vanRees et al., 2020), dispersing weeds continuously while harvesting. Given the spatial resolution of our GPS data, we could not study the microhabitats in which seeds were deposited at a fine scale within our models, and could not be certain when gulls were inside a field or on the dykes separating fields. Gulls egest particularly high densities of seeds on dykes, paths and field edges where they typically roost (Lovas-Kiss et al., 2018a). As reflected in the distribution of seed dispersal distances, LBBG disperse most seeds beyond the fields where ingestion occurred. Both the length and width of individual fields in our study area are typically <500m (see Google Earth), and hence below the median dispersal distance within ricefields (Table 1). Hence, if a new weed or a new herbicide resistant genotype appears in one field, LBBGs are capable of spreading it quickly across the entire ricefield area.

Seed dispersal into other habitats

The remaining 8% of the seeds were dispersed outside of ricefields (median dispersal distance 18 km) with maximum distances of 131-151 km, into a range of habitats potentially suitable for weed establishment. Around 42% of these seeds were deposited into moist or wet environments most likely to be suitable for modelled weed species (permanent irrigated habitats, along the river and in other waterbodies). However, some weeds dispersed by LBBG can establish in less moist habitats, especially those with Ellenberg moisture values below 6 (Table S1). This includes the alien *A. retroflexus*, which is ranked third in the list of alien invasive plants in China, due to negative impacts in wetlands and on agricultural production (Bai & Shang, 2017). The displacement of herbicide

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resistant weeds (e.g. *Cyperus difformis*, *Polypogon monspeliensis*, *Amaranthus retroflexus*) outside of the ricefields may have important economic implications in other crop types (Farmer et al., 2017), including the irrigated cotton, sunflowers and wheat in areas surrounding the ricefields.

The stretch of the Guadalquivir River that bisects the ricefields received particularly high densities of seeds and is tidal, so weeds deposited there by LBBG can potentially colonize habitats upstream to Seville as well as downstream towards the sea. The capacity of seeds egested by LBBG to disperse secondarily by hydrochory should be investigated. Aquatic plants such as duckweed and charophytes are also dispersed by LBBG feeding in ricefields, as are a range of aquatic invertebrates (Table S1, see also Lovas-kiss et al., 2018a). For these organisms, successful dispersal may occur to wetlands as far as Fuente de Piedra lake (118 km, Fig. 2), a major roosting site for the LBBG (**Chapter 1** and **4**). LBBG are likely to enable stepping stone dispersal between wetlands for a range of weeds and aquatic plants (and invertebrates). Many other wetlands outside Fig. 2 are interconnected by LBBG movements across Andalusia (**Chapter 1**).

We carried out our study at a regional scale and did not include migratory flights between breeding and wintering grounds, when considerably greater MDDs by LBBG would be expected (Viana et al., 2016c). Seed dispersal distances of 293-413 km were estimated for mallards (*Anas platyrhynchos*) during migratory flights based on GPS trajectories (Kleyheeg et al., 2019). LBBGs are likely to have an important role in long distance weed dispersal between Andalusia, Morocco and more northerly regions of Spain during their migrations (Shamoun-Baranes et al., 2017).

When departing the ricefields, gulls often visited three landfills (Fig. 2), which are foraging habitats and are also used for stopovers when LBBG move from one part of Andalusia to another (**Chapter 1**). In these cases, we cannot rule out the possibility that the gull individuals did not feed in ricefields during the hours immediately prior to flying to landfills, and that this may have led us to overestimate numbers of seeds dispersed along these trajectories. However, even if gulls had only been feeding in ricefields the day before they flew to landfills, the

maximum retention times recorded suggest they would still disperse seeds along the route connecting the landfill and ricefields. Faecal samples collected from Andalusian landfills provide further evidence that seeds are egested at these sites (authors, unpublished data).

Conclusions

It is vital to recognize the importance of waterbirds in endozoochory of vascular plants lacking a fleshy fruit, many of which are widely assumed to have no mechanisms for long-distance dispersal. This includes weeds with important implications for agricultural management, for which both humans and waterbirds are likely to be key vectors, but with different roles. Our study illustrates how seed shadows generated by waterbirds can be predicted. These vectors generate spatial dispersal patterns that are very different to those expected from recognized dispersal syndromes, demanding a revision of our understanding of plant dispersal processes. We found gulls to disperse seeds over greater distances than have been reported in seed shadows by frugivores. Similar spatial studies are needed in other waterbird-weed systems, and should ideally be integrated with studies of establishment success and population genetics of weeds growing in different habitats.

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Supplementary materials

Table S1. List of angiosperm species known to be dispersed by lesser black-backed gulls in Doñana ricefields. Data from **Chapter 2** and Lovas-Kiss et al., 2018a. Species used for the experimental retention curve are shown in bold.

Family	Taxa	Dispersal syndrome	Ellenberg value
Amaranthaceae	<i>Amaranthus albus</i> ^{a, b, c}	<i>Epizoochory</i>	5
	<i>Amaranthus retroflexus</i> ^{a, b, c}	<i>Epizoochory</i>	4
Asteraceae	<i>Senecio vulgaris</i> ^b	<i>Anemochory</i>	5
Araceae	<i>Lemna minor</i>	<i>Hydrochory</i>	11
Cyperaceae	<i>Cyperus difformis</i> ^{b, c}	<i>Barochory</i>	10
Euphorbiaceae	<i>Chamaesyce humifusa</i> ^a	<i>Myrmechocory</i>	2
Elatinaceae	<i>Bergia capensis</i> ^a	<i>Autochory</i>	-
Juncaceae	<i>Juncus subnodulosus</i>	<i>Epizoochory</i>	7
	<i>Juncus bufonius</i> ^b	<i>Epizoochory</i>	7
Poaceae	<i>Polypogon monspeliensis</i> ^{b, c}	<i>Epizoochory</i>	8
Polygonaceae	<i>Rumex dentatus</i>	<i>Hydrochory</i>	-
Portulacaceae	<i>Portulaca oleraceae</i> ^{b, c}	<i>Barochory</i>	2
Ranunculaceae	<i>Ranunculus sceleratus</i>	<i>Hydrochory</i>	9
Solanaceae	<i>Solanum nigrum</i> ^{b, c}	<i>Endozoochory</i>	5
	<i>Solanum dulcamara</i>	<i>Endozoochory</i>	8

^a Taxa alien to Spain according to AgroAtlas (2020), <http://agroAtlas.ru.>, ^b considered an agricultural weed, and ^c known to have herbicide resistant populations according to Heap (2020) and AgroAtlas (2020).

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Table S2. Sensitivity analyses for the seed dispersal model based on experimental retention times. Individuals with the highest average seed dispersal distances were removed sequentially in pairs. Wilcoxon test was used to compare differences between total model and alternative models when individuals were removed sequentially.

	Dispersal distance (Km)					Wilcoxon test
	Geometric Mean (95% CI)	25%	50%	75%	Max	
total	0.58 (0.56-0.60)	0.16	0.69	2.33	150.96	
2 individuals removed	0.57 (0.55-0.60)	0.17	0.68	2.28	130.62	W = 49818541, p= 0.65
4 individuals removed	0.58 (0.56-0.61)	0.18	0.68	2.28	135.59	W = 50011924, p= 0.97
6 individuals removed	0.57 (0.55-0.60)	0.17	0.69	2.21	135.81	W = 49794657, p= 0.61
8 individuals removed	0.57 (0.55-0.59)	0.16	0.68	2.28	135.74	W = 49668378, p= 0.41

Table S3. Detailed classification of habitat types outside ricefields, and numbers of seeds deposited in each according to the model based on experimental retention times. Land use was classified by the Junta de Andalucía in 2013.

Urban	305
Port	7
Mixed Urban	12
Industrial	47
Mining	43
Water facilities	6
Roads	10
Solid Waste treatment plant / landfill	180
Other water bodies	58
Industrial salt ponds	21
Marshland	9
Sea	2
Lakes and lagoons	4
Reservoirs	4
Agricultural ponds	18
River	68
Irrigated agriculture other than ricefields	183
Other agriculture	69
Olive groves	49
Vineyards	4
Citric crops	12
Other woody crops	8
Natural vegetation	40
Quercus	14
Eucalyptus plantations	5
Shrubland	2
Grassland	12
Bare ground	3
Dunes	4
Total	723

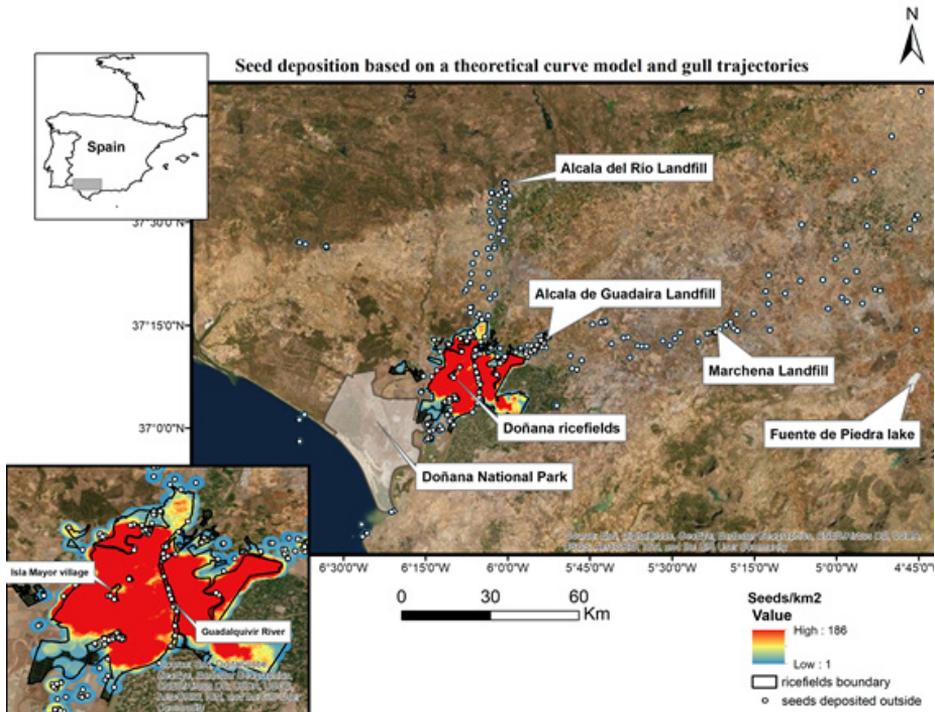
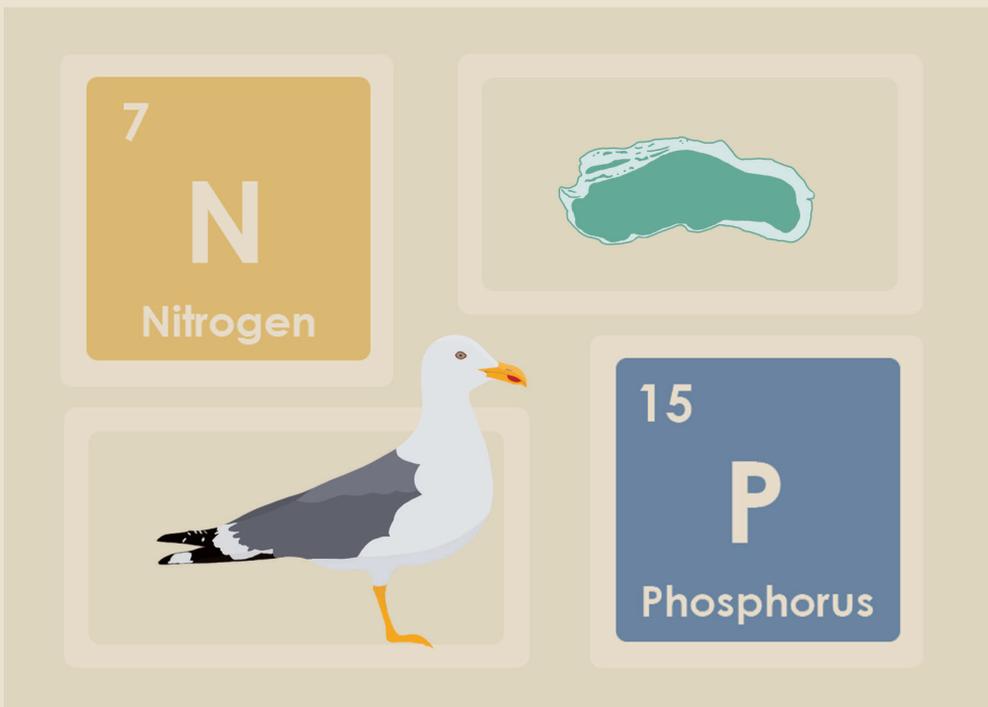


Figure S1. Point density distribution of 10,000 seeds whose dispersal was modelled based on a theoretical retention time curve and GPS trajectories. Red areas show sites where the highest density (maximum 186 seeds km⁻²) of seeds were deposited, which coincides with the ricefield area and its boundaries. Blue areas show lower densities of seed deposition. Each white dot shows one seed deposited outside ricefields. Seeds deposited in ricefields (90.7% of the total) are not shown individually.

Chapter 4

Quantifying nutrient inputs by gulls to a fluctuating lake, aided by movement ecology methods



Martín-Vélez V., Sánchez M. I., Shamoun-Baranes J., Thaxter C. B., Stienen E. W., Camphuysen K. C., & Green A. J. (2019). Quantifying nutrient inputs by gulls to a fluctuating lake, aided by movement ecology methods. *Freshwater Biology*, 64(10), 1821-1832.

Abstract

Eutrophication of aquatic ecosystems is a global problem with major ecological and economic impacts. In many lakes and reservoirs, guantrophication occurs when roosting waterbirds import nutrients (nitrogen and phosphorus) from surrounding terrestrial habitats. To date, nutrient loading by waterbirds has been estimated based on censuses in the absence of detailed information on their movements. We quantified nutrient importation by the lesser black-backed gull (*Larus fuscus*) to Fuente de Piedra (1350 ha) in Andalusia (south-west Spain), where an average of 36,288 individuals are counted in January.

During seven winters from 2010 to 2017, we used movement data from 20 individual gulls tagged with GPS trackers that foraged in four landfills. Together with monthly bird counts and measurements of Total N and P content in faeces and pellet samples, movement data were used to quantify the total external loading effect for different winters. Movement data allowed us to quantify the proportion of time spent in the lake and the time spent at different foraging sites, and enabled correction of censuses.

According to tracking data, on average 69% of the birds had already left the lake to head for feeding sites when waterbird counts were carried out. Nutrient inputs to the lake depend partly on the proportion of the day that gulls spend there, which was higher in late winters and was reduced when lake depth went below or above 20-35 cm. An estimated average of 10.17 Kg N ha⁻¹ y⁻¹ and 2.07 Kg P ha⁻¹ y⁻¹ were imported to this closed-basin lake by gulls each winter, with highest values recorded in winter 2016-2017.

Gull guano is the most important winter source of nutrients to the lake. Regurgitated pellets have been ignored as a source of nutrients in other guantrophy studies, but we found them to be a more important source of P than faeces. A movement ecology approach complements traditional censuses and facilitates the study of guantrophication in multiple ways, including identification of sources of nutrients, correction of censuses, and measuring time spent at roost sites.

Introduction

Eutrophication of aquatic ecosystems is a serious environmental problem worldwide (Harper, 1992; Carpenter, 2005) and is one facet of the Global Water Crisis (Mateo-Sagasta et al., 2017). In the European Union, nearly 40% of water bodies are affected by eutrophication due to agricultural pollution (WWAP, 2015), and many others are affected by inputs of urban wastewaters (Vymazal, 2010; de-los-Ríos-Mérida et al., 2017). The need to reduce eutrophication has led to a number of EU Directives (Knockaert, 2014 a, b; van Buuren, 2014). Eutrophication can lead to excessive plant productivity, harmful algal blooms, proliferation of floating plants, anoxic events and fish mortality, with major impacts on diversity and food web structure (Bauer & Hoyer, 2014; Vizzini et al., 2016). The impact of eutrophication is expected to increase in coming years due to the increase in human population, land use conversion, soil erosion and fertilization (Millennium Ecosystem Assessment, 2005) and the consequences of climate change (Hanjra & Qureshi, 2010; Green et al., 2017).

While most studies have focused on human-mediated eutrophication (i.e. cultural eutrophication), guantrophication (i.e. animal-derived fertilization) can also be important. This typically occurs when large numbers of birds feed elsewhere, but roost or nest in lakes or reservoirs and is particularly problematic in closed-basin lakes (i.e. those with no outflow). Birds can be key biovectors of nutrients and have important effects on ecosystem functioning (Green & Elmer, 2014; Dessborn et al., 2016). Waterbirds produce faeces that are rich in phosphorus (P) and nitrogen (N), and omnivorous birds such as gulls (Laridae) have higher protein content in their diets than herbivorous birds such as geese, leading to higher P loading into roost sites. This in turn promotes harmful algal blooms and the loss of submerged plants (Marion et al., 1994). Guantrophication by gulls that use landfills for foraging then roost in lakes or reservoirs is a widespread problem, causing damage to ecosystem services estimated at \$100 million in North America alone (Winton & River, 2017).

Guantrophy studies rely partly on estimates of body mass functions and daily food intake to model the nutrient loading to wetlands (Hahn et al., 2007, 2008).

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These models also rely on waterbird counts at wetland roosts and assumptions about the proportion of time that birds spend in the wetland, which has a direct influence on the proportion of daily faecal output egested there (Hahn et al., 2008; Winton & River, 2017). However, tracking daily movements of individuals allows the quantification of time use and movements between feeding and roosting sites that can then complement censuses and help to quantify nutrient loading. GPS tracking also allows identification of specific feeding sites (e.g. landfills) which act as a source of the nutrients imported to wetlands, as well as of contaminants (e.g. heavy metals) or pathogens (Bauer & Hoye, 2014).

In Andalusia, the lesser black-backed gull (LBBG) *Larus fuscus* has become an important wintering waterbird on inland waterbodies, due to a major increase of the European breeding population since the middle of the twentieth century (Hagemeijer & Blair, 1997; Wetlands International, 2019). The LBBG has also undergone roughly a tenfold increase in numbers since the 1970's in Andalusia (Rendón et al., 2008), and is now the second most numerous wintering waterbird, after the Northern Shoveler *Anas clypeata* (census data from the Junta de Andalucía). This increase is probably related to the expansion of anthropogenic habitats such as rice fields (which have doubled in surface area since the 1960's, Ramo et al., 2013) and landfills (Fig. 1, and Arizaga et al., 2018) that increase resource availability. Communal roosting by LBBG at wetlands is common, thereby reducing thermoregulatory costs and predation risk (Galván et al., 2003). This behaviour, along with its abundance and the availability of GPS-tracking data, make the LBBG an ideal study model to apply movement data to evaluate avian inputs of nutrients into wetlands. Here, we use the most important mid-wintering site for LBBG in Andalusia as a case study. Fuente de Piedra is a shallow, hypersaline (average salinity 41.2 g L⁻¹, Rodríguez-Rodríguez et al., 2010), closed-basin lake and a protected wetland famous for its waterbirds.

We test the following hypotheses: 1) movement data allow us to quantify the proportion of the day birds spend at Fuente de Piedra, and hence the proportion of daily excreta likely to be deposited there; 2) changing water levels in the lake lead to variation in gull numbers and the proportion of time spent each day at the lake, within and between winters. Low water levels would expose gulls to

predators such as foxes, whereas very high water levels would inundate islands in the lake where gulls roost (Fig. 1C and Bijleveld et al., 2010). Therefore, we expect higher gull numbers and longer roosting time at intermediate water levels; 3) movement data allow us to identify different open landfills used as feeding sites by the gulls roosting at Fuente de Piedra, and allow us to quantify their relative importance for nutrient loading; 4) regurgitated pellets represent an important fraction of the contribution of LBBGs to the nutrient budget of Fuente de Piedra. Pellets are produced regularly by LBBG at roost sites (Lovas-Kiss et al., 2018a), yet have been overlooked in previous guanotrophy studies (Winton & River, 2017).

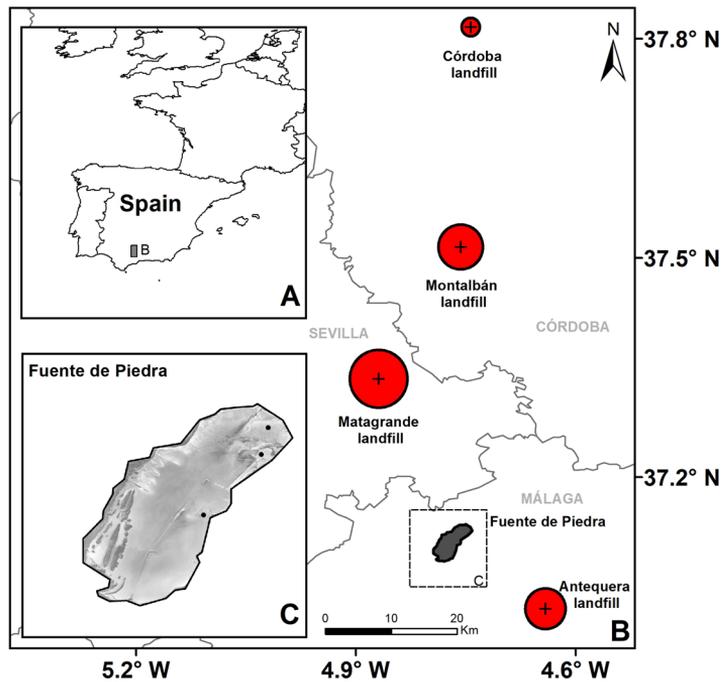


Figure 1. (A) Location of Fuente de Piedra shallow lake in the Iberian Peninsula. (B) Four landfills used as foraging sites by gulls making daily movements from Fuente de Piedra. The size of the circle is proportional to the total cumulative time gulls spent in each site between 2010 and 2017. Matagrande landfill was opened in 2006, Antequera in 1993, Montalbán in 1992 and Córdoba in 1986. (C) Dots show the location of the main roosting sites of gulls within Fuente de Piedra.

Material and Methods

Study area

This study was performed in Fuente de Piedra (FP), a shallow lake located in Malaga province (south-west Spain; 37° 6' N, 4° 44' W), which is protected at regional (Natural Reserve), European (Special Protection Area) and international (Ramsar site) levels (Fig. 1). It is the most important breeding site for greater flamingos (*Phoenicopterus ruber*) in the Iberian Peninsula, with around 10,500 breeding pairs (Bechet et al., 2012). LBBG are now abundant at FP in winter, but January counts did not exceed 40 individuals prior to 1988 (M. Rendón Pers. Comm.). Fuente de Piedra is the largest natural lake in Andalusia and is situated in a closed basin of karstic origin covering an area of 1,350 ha (6.8 km long and 2.5 km wide) (Batanero et al., 2017) at 400 m above sea level (García et al., 1997). Inputs of water come from rainfall, ground water and two intermittent streams, whereas output is mainly due to evaporation (Rodríguez-Rodríguez et al., 2005). Fuente de Piedra fluctuates in water level, salinity and nutrient concentrations, tending to increase in depth during the course of the winter (Fig. 2B) but drying out in summer in most years, although it retains water throughout wet years (Rodríguez-Rodríguez et al., 2005). It is a hypersaline lake of the Cl–(SO₄)–Na–(Mg)–(Ca)-type, and concentrations of dissolved solids vary between 18 g L⁻¹ and 200 g L⁻¹ (Kohfahl et al., 2008). Salinity varies seasonally, ranging from 10 g L⁻¹ to a maximum in summer of 400 g L⁻¹ (Benavente et al., 2003). In wet and dry years, Total Nitrogen (TN) ranged on average from 0.22 to 0.59 mmol N L⁻¹; Total Dissolved Nitrogen (TDN) from 0.18 to 0.28 mmol N L⁻¹; Total Phosphorus (TP) from 4.85 to 12.61 μmol P L⁻¹ and Soluble Reactive Phosphorus (SRP) from 0.45 to 0.74 μmol P L⁻¹ (Batanero et al., 2017). Daily water level measurements were provided by the Junta de Andalucía (regional government), and taken using a limnigraph that registers water level variations through movements of a floating sensor located in an open shallow well in the lake.

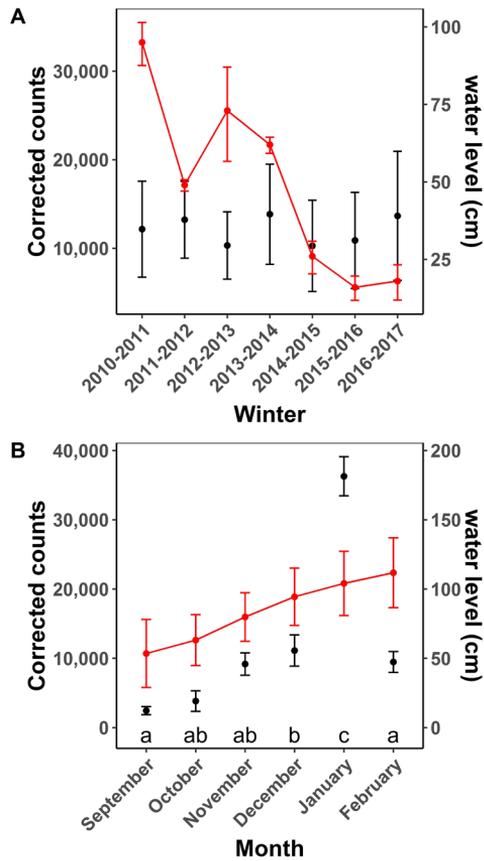


Figure 2. (A) Plot of corrected counts (mean \pm s.e.) in Fuente de Piedra between 2010 and 2017, based on six monthly counts per winter. The mean (\pm s.e.) water level per winter is shown in red, based on daily measures. (B) Monthly seasonal variation in corrected counts (mean \pm standard error) in Fuente de Piedra between 2010 and 2017. Significant differences ($p < 0.05$) between months in the number of gulls in Fuente de Piedra lake are represented by different letters below error bars, based on a Tukey post-hoc test. The mean (\pm s.e.) water level per winter is shown in red.

GPS tracking

We used GPS tracking data collected as part of long term studies of several breeding populations using UvA-BiTS (Thaxter et al., 2015; Shamoun-Baranes et al., 2017; Baert et al., 2018), (<http://www.uva-bits.nl>; Bouten et al., 2013). We first selected all data points from a centralized database that fall spatially within the boundaries of FP from September through February (i.e. the wintering period) in 2010-2017 (i.e., seven winters). We applied a buffer zone of 50 meters to account for individuals roosting around the lake edge. The resulting dataset included tracking data from 20 individuals. Eight individuals originated from the Zeebrugge colony (Belgium), five from Texel (the Netherlands), three from Walney (UK), three from Skokholm (UK) and one from Orfordness (UK). In winter, the trackers recorded GPS movements at intervals of 10-30 minutes. Each individual was recorded at FP for one to six different winters, and in total we had data from 29 bird-winters (Fig. 3).

For our study, we only considered daily trips in which the tagged individuals departed from FP and came back to roost the same day. Each GPS point was assigned a duration (min) based on the backward and forward intervals between consecutive GPS points providing a “centred duration”. We removed gaps in the data which had a centred duration of more than 60 min. The number of GPS fixes per day varied between individuals and during the course of the winter, so we calculated the percentage of daily time spent at FP on a given day as the accumulated minutes for the fixes whilst residing at FP, divided by the total accumulated minutes for all fixes that day. Days in which the position of individuals was known for less than 1,000 minutes per day (e.g. because of missing data) were discarded. After data visualization, we identified four landfills as foraging destinations (Fig. 1). The accumulated time spent (in minutes) of the fixes that fell spatially within the boundaries (determined from Google Earth Satellite images) of each landfill was calculated for each day when a gull roosted at FP.

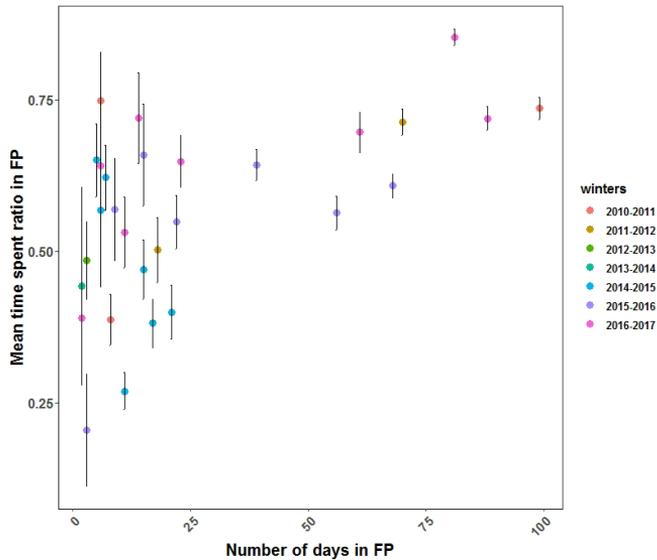


Figure 3. Proportion of daily time spent ratio (mean + s.e.) in Fuente de Piedra per winter for tagged gulls over seven different winters, in relation to the number of days spent at the lake the same winter. When considering data for all years combined and the proportion of daily time as dependent variable, there was a significant relationship with the number of days that individuals spent at FP ($F_{1,28} = 12.96$, $p = 0.001$). When controlling for years with a factor, the year effect is not significant whereas the relationship with number of days remains significant ($F_{1,22} = 11.69$, $p = 0.002$).

Census estimation and analysis

Monthly censuses (from September to February) during seven “winters” from September 2010 to February 2017 were carried out at FP by the Junta de Andalucía, but no counts were made at foraging sites. Bird counts were performed between 08:00 a.m. and 12:00 p.m. local time, but the precise time on each occasion was not recorded (M. Rendón Pers. Comm.). Missing counts (5 out of 42 months; 11%) were imputed based on the type III Poisson Regression trend by using the *RTRIM* package in R (Van Strien et al., 2004). Using GPS movement data, we determined the departure times (converted from UTC to local time) of all gull-trips ($N=374$) from FP after nocturnal roosting for all available data during the seven years of study. For each interval of five minutes between 08:00 h. and 12:00

h, we calculated the proportion of individuals that roosted during the previous night which still remained at FP. In this way, we estimated the proportion of gull-trips that had not yet started, enabling correction of counts at the lake.

Variation in time spent at the lake

We tested if the proportion of time spent at the lake on a given day was related to the number of days spent there. We performed a Linear Model with “number of days” and “winter” as response variables and “daily proportion of time spent” (logit transformed) as dependent variable. Next, to evaluate the effect of a specific “winter”, water level, and date within the winter season on the “daily proportion of time spent” at the lake, we performed a generalized linear mixed-effect model (GLMM) with binomial error distribution and logit link function. “Day” of the year and “water level” were included in the GLMM as a second-order polynomial, in order to allow for non-linear relationships through the wintering season. To reduce collinearity between “day” and “day squared” (Legendre & Legendre, 1998), the first of January was set as day 0 and the days from September to December were given negative values. For the same reason, water level was first transformed by deducting the mean from all values, and then added as a second order polynomial. The complete model included “winter” as a fixed factor with seven levels, and “water level”, “water level squared”, “day”, and “day squared” as continuous predictor variables. The “daily proportion of time spent” roosting at FP was the dependent variable. We first included “individual” as random factor, but it only explained 0.0001% of the variation of the data (because different winters generally featured different individuals), so it was finally removed from the model. Akaike Information Criterion (AIC) model selection was used through the *drop1* function in R in order to gradually drop variables from the complete model (AIC=741.20) until a minimum adequate model was reached. The final model (AIC=737.91) had “winter”, “water level” and “water level squared” as predictor variables. The fit of the final model was assessed by the ratio between the residual deviance and the number of degrees of freedom (the ideal ratio being one; Crawley, 1993). Main effects were tested by comparing the final model with an alternative model without the variable to be tested. Post-hoc tests for the differences between winters were performed with

a Multiple Comparisons of Means (Tukey Contrast with Holm adjustment). The non-linear relationship between water level and the proportion of time spent at the lake was fitted via a Loess smooth regression.

Variation in time spent at foraging sites

To test for differences in the “daily time spent” per visit at each of the landfills used as foraging sites (daily accumulated time, log transformed to normalize residuals), we performed a Linear Mixed Model (LMM) with a normal error distribution and Gaussian link function, including “site” and “winter” as fixed factors, and “individual-ID” as a random factor. Post-hoc tests for differences between foraging sites were performed as above.

Variation in numbers of gulls at the lake

To determine if gull imputed counts varied between different months or in response to changing water level, we performed a General Linear Model (GLM) with quassipoisson error distribution and log link function. We selected “winter” and “month” as factors with seven levels and six levels respectively, and “water level” and “water level squared” as continuous variables.

All analyses were performed in R (v.3.4.1) using packages *lmerTest* (Kuznetsova et al., 2015), *lme4* (Bates et al., 2014), *multcomp* (Hothorn et al., 2017) and *blme4* (Korner-Nievergelt et al., 2015).

N and P content in gull excreta

Ten fresh gull faecal samples and ten fresh pellets were collected in FP in February 2017 and placed in ice in the field, then frozen at -20°C within 3 hours to prevent ammonium volatilization. Samples were taken from three different roosting sites separated by up to 2 km (see Fig. 1C), and within each site were collected from spots separated by at least one meter to ensure they were from different individuals. Samples were later dried at 60°C during 24 hours to obtain dry weight. Pellets were triturated into dust in a mixer mill (Retsch MM 400)

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during 3 minutes at 230 Hz. We then diluted 0.5 g in 500 mL of Milli-Q water and stored it at -20 °C until analysis in April 2017 (for faeces) and October 2018 (for pellets). We analysed Total Nitrogen (TN) by digestion with potassium persulfate (Nydahl, 1978). Total Phosphorus (TP) concentration was measured using the molybdenum blue method (Murphy & Riley, 1962). The Coefficient of Variation ($CV = (\sigma / \mu) * 100$) was calculated for each sample type and nutrient type.

Nutrient quantification.- Total Nutrient Loads (NL) of LBBG per winter were estimated as follows (the same equation can be used for either N or P) :

- (1) $NL = NL_{faeces} + NL_{pellets}$
- (2) $NL_{faeces} = TS * ER_{faeces} * ND * MW * NC_{faeces}$
- (3) $NL_{pellets} = ER_{pellets} * PW * ND * MW * NC_{pellets}$

where TS= Time Spent; ER= Excretion Rate; PW= Pellet Weight; ND= Number of Days per winter; MW= Mean Winter counts and NC= Total Nutrient (either N or P) content.

TS= average daily proportion of Time Spent at the lake per individual per winter.

ER_{faeces} = Excretion Rate (ER) per individual per day ($g\ day^{-1}$), considered as a fixed parameter ($21.06\ g\ day^{-1}$), calculated using equation 3 of the Hahn et al. (2007) model for nutrient transport by carnivorous birds. For these calculations, we assumed that LBBGs have a mean body mass of 792 g (Hahn et al., 2007) and a daily energy requirement of 968.66 kJ (Nagy et al., 1999). We adopted values of food energy content of $23.9\ kJ\ g^{-1}$ based on landfill diet (Winton & River, 2017), a metabolizable energy coefficient of 0.76 (Karasov, 1990), and an intake: excretion ratio of 0.395 (Dobrowolski et al., 1993; Nixon & Oviatt, 1973).

$ER_{pellets}$ = Egestion Rate (ER) of pellets per day. One pellet is assumed to be produced per day (Lovas-Kiss et al., 2018a), and deposited within the roosting site.

PW= mean Pellet Weight (PW). Based on the average dry weight of pellet samples collected ($3.74 \text{ g pellet}^{-1} \pm 1.18 \text{ s.d.}$).

ND= Number of Days (ND) per winter (180 days, for a wintering period from September to February inclusive).

MW= Mean Winter (MW) count of LBBG after we corrected the counts for gulls that were missing (i.e. those that had already set off for feeding sites by the time the lake was counted).

NC_{faeces}= average Total Nutrient (either N or P) in grams per gram of faeces.

NC_{pellets}= average Total Nutrient (either N or P) in grams per gram of pellet.

We also compared our results to those predicted from previous literature that do not include movement data. Following Hahn et. al (2007), we took TS as a fixed value of 0.6, and calculated MW directly from counts without correcting for birds that had already left the lake.

Results

Gulls predominantly used the lake for nocturnal roosting. In a small minority of gull-days (30 of 786 gull-days [3.8%] during winters from 2010 to 2017), tagged gulls remained at FP all day without leaving to feed elsewhere.

Evaluating errors in censuses

Using the departure times of GPS-tagged gulls from FP, we estimated that on average only 31% of the gulls were present at the lake when counts were made (decreasing from 59% at 08:00 h to 26% at 12:00 h in intervals of 5 minutes, Fig. 4). Using this proportion (0.31) we estimated the number of gulls that were present at FP from 2010 to 2017 (Fig. 2A), as well as the fluctuations in their abundance across months (Fig. 2B). A General Linear Model of corrected count data showed that gull numbers varied significantly among months (model

comparison: $F= 22.312$, $d.f.= 5$, $p< 0.0001$). Gull numbers were significantly higher at FP in January than other months (Fig. 2B).

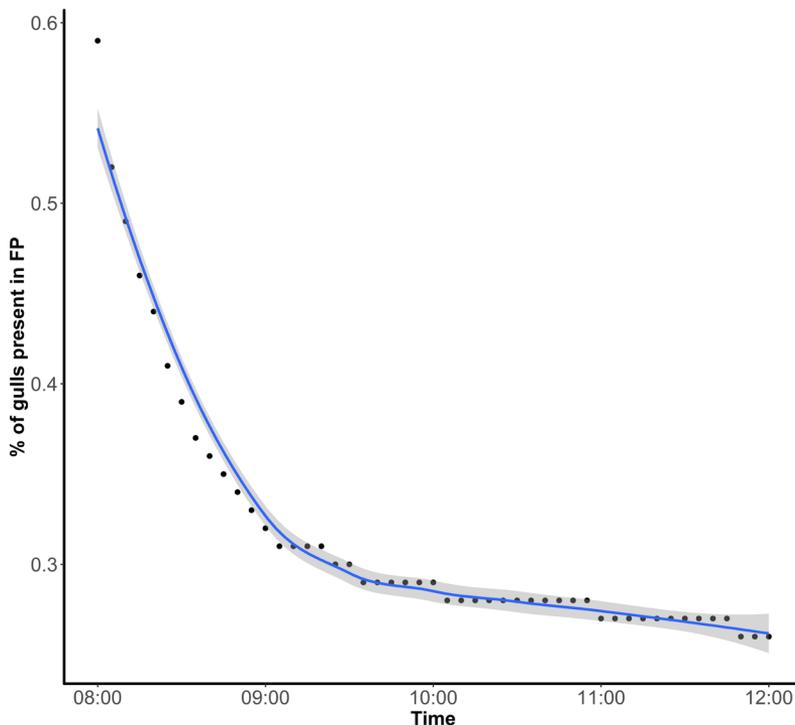


Figure 4. The proportion of tagged gulls (based on 374 gull-trips) that remained in Fuente de Piedra lake during the time range when gull counts were conducted, in intervals of 5 minutes. Those gulls not present at the lake had already left for the foraging sites.

Time spent by gulls in Fuente de Piedra

Within a single winter, individual gulls varied in the proportion of the day spent at the lake (Fig. 3). Furthermore, data were available for a smaller number of tagged gulls during the four first winters than in the last three winters (range from 1 to 8 individuals, Fig. 3). In addition, individuals that spent more days at FP also tended to spend a greater proportion of the day there (Fig. 3).

For the proportion of daily time spent at the lake, the final model with parameters “winter”, “water level” and “water level squared” provided a good fit to the data (ratio of 0.92; deviance= 717.9, d.f.= 776). Model comparison showed that the “winter” factor had a significant effect on time spent by gulls in the lake ($X^2=12.84$, $p=0.046$). Comparison between winters showed that gulls spent most time per day in the lake during winter 2016-2017, followed by 2015-2016 and 2014-2015 respectively (Fig. 5). Water level did not significantly influence the time gulls spent in FP when combined with winter ($X^2=2.97$, d.f.= 2, $p=0.228$), but it did when analysed on its own and in a non-linear manner ($Z=-3.958$, d.f.= 783; $p<0.0001$) with most time spent when water levels were close to 30 cm (Fig. S1).

Location of foraging sites and time spent there

Based on the GPS tracks, the feeding sites used by LBBG when roosting at FP were the landfills at Antequera (17.8 km from FP), Matagrande (26.7 km), Montalbán (44.7 km) and Córdoba (78.3 km, Fig. 1). Matagrande landfill was the site where gulls spent most accumulated time, followed by Montalbán and Antequera landfills (Fig. 1). According to a Linear Mixed Model, “site” had a significant effect on the time spent at the foraging site per visit ($F_{3, 914.71}=33.94$, $p<0.0001$), whereas “winter” did not ($F_{6,159.70}=2.1$; $p=0.055$). Gulls spent significantly less time per daily visit at Antequera landfill, which was the closest foraging site and the one visited most often (Fig. 6).

Nutrient loading quantification

Mean TP in faecal samples was $1.82 (\pm 0.86 \text{ s.d.}; \text{CV}=47.6\%) \text{ mg P g}^{-1}$ dry mass, whereas mean TN was $18.86 (\pm 6.55 \text{ s.d.}; \text{CV}=34.7\%) \text{ mg N g}^{-1}$ dry mass (Fig. 7). Therefore, the N:P ratio in faeces was approximately 8:1. Mean TP in the pellet samples was $5.68 (\pm 4.86 \text{ s.d.}; \text{CV}=85.5\%) \text{ mg P g}^{-1}$ pellet whereas mean TN was $10.90 (\pm 3.39 \text{ s.d.}; \text{CV}=31.1\%) \text{ mg N g}^{-1}$ pellet (Fig. 7), so the N:P ratio in pellets was approximately 1.9:1. Based on means for nutrient content, % time spent in the lake per winter (September-February 2010-2017) and corrected censuses (together with fixed parameters such as excretion rate and number of

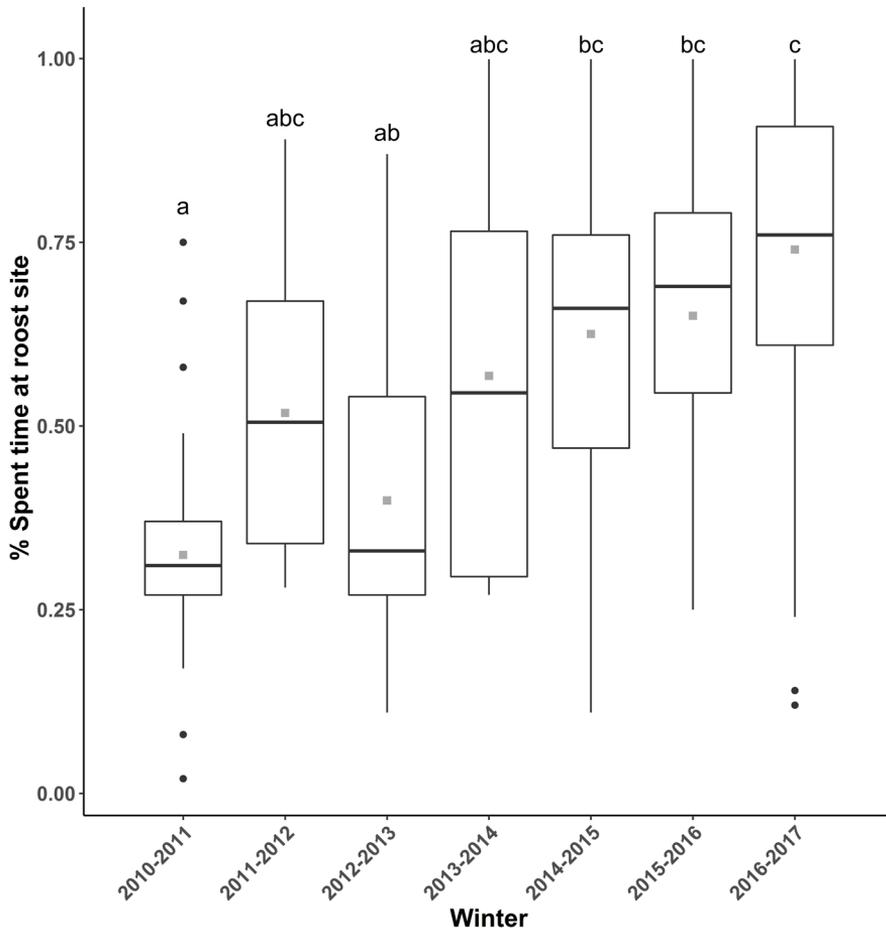


Figure 5. Proportion of daily time spent at the roost (Fuente de Piedra) in winters from 2010 to 2017. Median values with quartiles are presented, with the arithmetic mean shown by grey squares. No shared letters above bars indicates a significant difference ($p < 0.05$) between years, based on a Tukey post-hoc test.

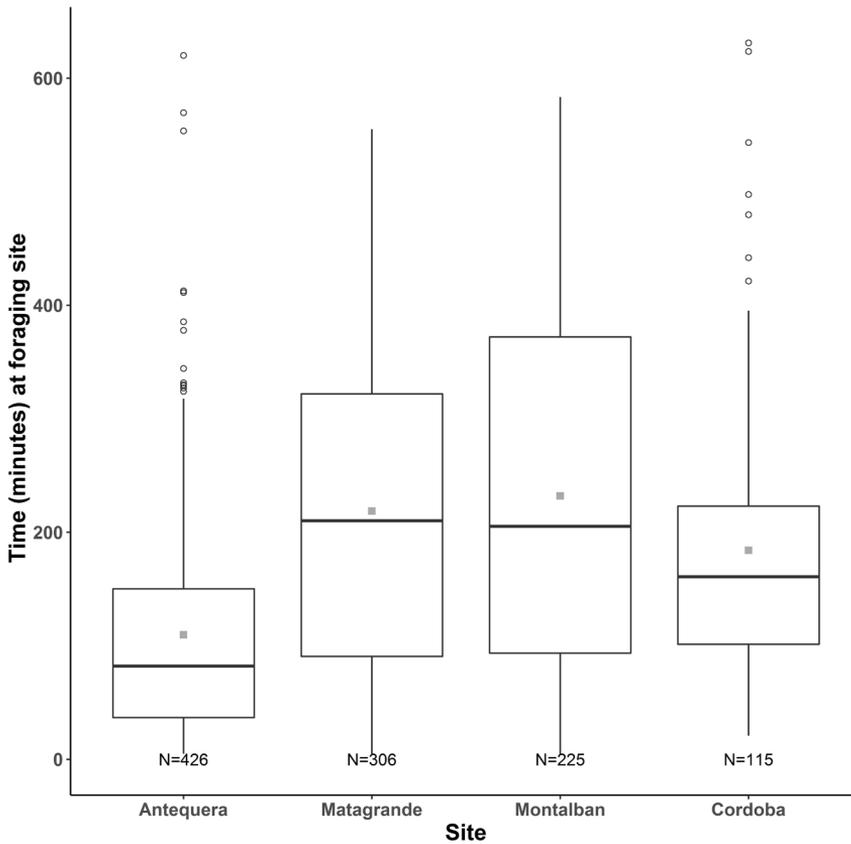


Figure 6. Time spent (in minutes) per daily visit in the foraging sites by gulls roosting at Fuente de Piedra from 2010-2017. Sites are in order of increasing distance to FP from left to right. Median values with quartiles are presented, with the arithmetic mean shown by grey squares. A Tukey post-hoc test from a linear mixed model revealed a significant difference between Antequera and all other sites ($p < 0.05$). N = Numbers of visits per foraging site. Note the difference with Figure 1, which shows the total time spent at each site when summing all visits together.

days per winter), we determined the TN and TP loading by LBBG into FP for both faeces and pellets (Fig. 8). Average annual total N and P loads per ha of gulls into FP amounted to 10.17 Kg N ha⁻¹ y⁻¹ and 2.07 Kg P ha⁻¹ y⁻¹ respectively, but varied between years (Fig. 8). Although faeces were more important than pellets as a source of imported N, pellets were more important than faeces as a source of P (Fig. 7).

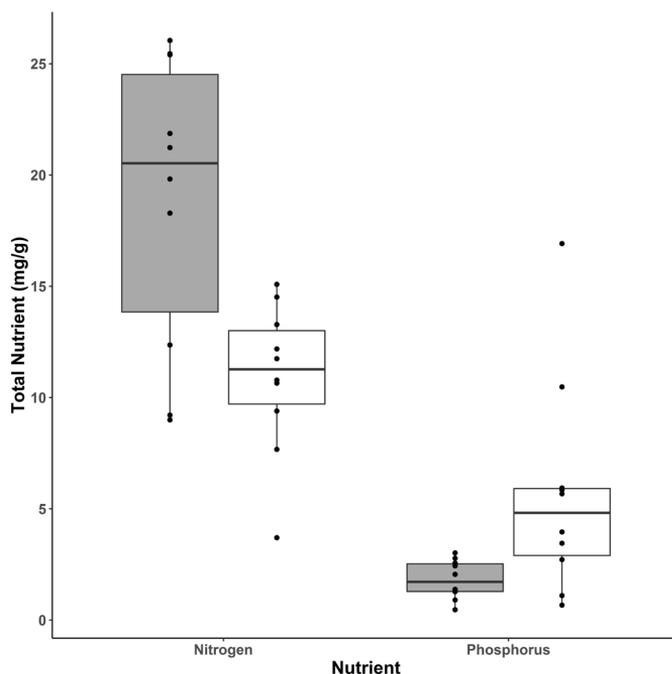


Figure 7. Total N and total P content of *Larus fuscus* droppings in grey (n=10) and pellets in white (n=10) from Fuente de Piedra lake.

Our estimates of nutrient inputs based on GPS movement data were compared with those based on the models of Hahn et al. (2007). Although there was strong variation between winters, on average our estimates of nutrient imports were 47% higher than those based on Hahn et al. (2007), partly because gull counts underestimate the number of birds present, and partly because the true proportion of the day spent at the lake was on average higher than the 60% estimated by Hahn et al. (2007) (although in some winters it was lower, Fig. 5).

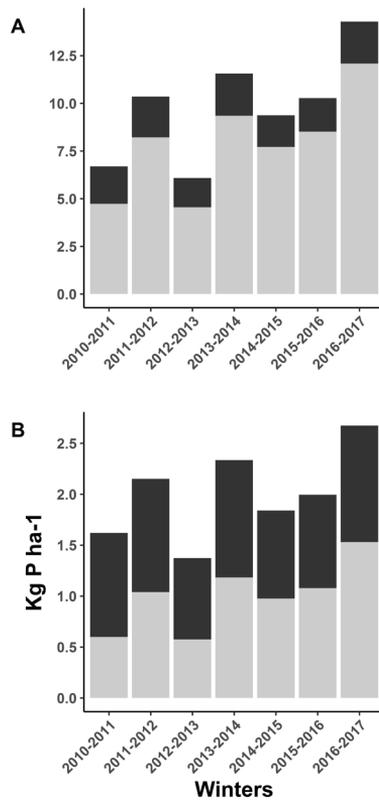


Figure 8. Estimated loading (Kg ha⁻¹) for (A) Nitrogen and (B) Phosphorus in Fuente de Piedra by *Larus fuscus* from faeces (light grey) and pellets (black) for each winter from 2010 to 2017.

Discussion

As far as we know, this is the first study to take advantage of detailed information from GPS data on time use and behaviour of waterbirds to complement waterbird counts and so estimate nutrient inputs by waterbirds with more confidence. All four of our initial hypotheses were accepted. We recorded substantial inputs of N and P by wintering gulls into a Mediterranean shallow lake, and found that these inputs increased in recent winters and are dependent on lake water levels.

By using GPS tracking data, we also identified the relative importance of different landfills which are the sources of nutrients imported to the lake, and which ultimately may have fuelled the increase in the gull population and in guano inputs over time. The landfills used by LBBGs roosting at Fuente de Piedra lake (FP) were created between 1986 and 2006, a period which coincides with the increase in the numbers of LBBG wintering in Andalusia. Similar effects have been observed in North America, where the creation of landfills has resulted in increased gull populations and guanotrophic effects (Winton & River, 2017).

Previous studies that estimate nutrient contribution by waterbirds lack detailed behavioural information, and use fixed parameters to estimate the time spent at the roosting site without accounting for variation within or between winters (Hahn et al., 2007, 2008; Dessborn et al., 2016) or correcting censuses for birds that were away from the roost site. When comparing nutrient loading estimations with and without use of movement data, we found an average increase of 47% when GPS data were used. This is mainly due to the effect of underestimating bird numbers when counting, and suggests that gull impacts may have been underestimated in previous guanotrophy studies (e.g. Winton & River, 2017).

Numbers of gull present

By using GPS movement data, we showed that approximately 69% of the birds were not present in FP during the times of day when counting was conducted. Unfortunately, we have no way of knowing if the error in counts varied seasonally or between years, but we cannot rule out this possibility (e.g. if the delay between

sunrise and counting changed during the course of the winter), which in turn would influence our estimates of nutrient inputs. This underlines the importance of recording the timing of waterbird counts with precision.

The highest number of LBBGs was recorded in January. During the first half of the winter when numbers were relatively low at FP, LBBG concentrate in rice fields in the Doñana area 150 km to the west, to feed on alien crayfish, waste grain and other food during the harvest (Rendón et al., 2008; Lovas-Kiss et al., 2018a). From November onwards, individuals disperse around Andalusia, using FP mainly during the second half of the winter. In January, FP holds 27% of the total number of LBBG counted in Andalusia (Junta de Andalucía data).

Gull activity in Fuente de Piedra

Gull tracking revealed that time spent at the roost site is not a fixed parameter but is instead subject to considerable variation in behaviour between individuals. More research is required to investigate why some birds stay at the lake longer than others, and how this relates to variation in the quality of roost sites. Clearly, increasing the number of tagged individuals is desirable, so that average values for time spent at FP are more reliable, and to throw further light on how and why different individuals contribute unequally to guano-trophication.

According to movement data, gulls also increased the number of hours a day they spent at FP in more recent winters, with the greatest proportion of time spent during the winter of 2016-2017. This trend may be partially driven by changes in the water level, since levels were lower in recent winters. The time spent at the roost site was highest when water levels within the lake were around 30 cm.

Gull activity at foraging sites

Our study reveals a massive translocation of nutrients from landfills to a natural, protected lake by roosting gulls (see Winton & River 2017 for similar examples in North America). Gulls spent most time at the Matagrande and Montalbán landfills (both in terms of total time and time per visit) at an intermediate

distance. Despite the much greater distance from FP to the Córdoba landfill, the time spent there per visit was as high as at the Matagrande and Montalbán landfills, although the total number of visits was lower. In contrast, gulls visited the closest landfill at Antequera more frequently but spent less time there per visit. Hence, distance seems to play a role in determining the number of visits to each foraging site, and LBBG compensate for travel costs to a more distant foraging site by spending more time there (see also Arizaga et al., 2010). Movement data also show that individual LBBG frequently switched between the four foraging sites within and between winters (results not shown), probably in relation to changes in the quantity of food available (Arizaga et al., 2014).

Variation in management procedures and protocols may determine the quantity and quality of food at each landfill, but new national directives concerning refuse management may change gull behaviour in the future. A new National Framework Waste Management Plan (Plan Estatal Marco de Gestión de Residuos, PEMAR 2016-2022), based on the Landfill Management Directive, was approved in 2015 for the period from 2016 to 2022. This directive requires the gradual reduction of biodegradable waste to 35% in 2016, with a further reduction of 35% in 2020, as well as measures to improve waste separation and recycling. Those initiatives are likely to reduce resources available to gulls. In combination with measures to deter gulls from foraging in key landfills (Castege et al., 2016), such measures could potentially reduce the number of gulls wintering at FP, and hence their guantrophication effects.

Quantification of nutrient loading

We found an increase of external nutrient loading by gulls in FP in more recent winters, related to an increase in both the proportion of time spent at the lake and the number of gulls wintering at the lake. Although our estimations are aided by precise information on gull movements, they also depend on other parameters which we did not directly measure. For example, our calculations assume a fixed defecation rate, while this may vary with diet (Dessborn et al., 2016). We used our own measures of the nutrient content of excreta, but our results were highly variable between samples. There were also high levels of variation within and

between previous studies of N and P concentrations of excreta (Winton & River, 2017), and overall results are highly sensitive to the nutrient concentrations used in loading calculations. Previous gull studies reported average concentrations of 68.6 mg g⁻¹ of N and 4.3 mg g⁻¹ of P in faeces (i.e. 4.8 and 2.3 times higher than our mean value respectively; Winton & River, 2017). Hence our calculations may underestimate the true loading rates by gulls at FP. On the other hand, pellet concentrations showed high P levels with much individual variation, and we included pellets in our loading calculations, unlike previous authors (Hahn et al., 2007; Winton & River, 2017). It is also noteworthy that studies of guanotrophy have not taken into account additional nutrient inputs from feathers (Williams & Berruti, 1971), which may be important (Fig. S2).

Relative importance of gull nutrient inputs compared to other nutrient sources

Expressed in hectares, the average nutrient inputs by the gulls in FP amount to 10.17 Kg N ha⁻¹ y⁻¹ and 2.07 Kg P ha⁻¹ y⁻¹ (Figure S3). Total nutrient load by flamingos at FP was estimated as 16.7 Kg N ha⁻¹ y⁻¹ and 1.24 Kg P ha⁻¹ y⁻¹, but these inputs are concentrated during the summer months when flamingos breed (Batanero et al., 2017). Atmospheric inputs were estimated as 5.89 Kg N ha⁻¹ y⁻¹ and 0.18 Kg P ha⁻¹ y⁻¹ for the study region (Morales-Baquero et al., 2013). A stream associated with the water treatment plant in the nearby town reportedly discharges 1.05 Kg N ha⁻¹ y⁻¹ and 0.28 Kg P ha⁻¹ y⁻¹ (de-los-Ríos-Mérida et al., 2017). Flamingo inputs thus seem to be the main source of N in FP (Fig. S3), but these estimates were made in the absence of movement data and inflated by an unrealistic assumption that flamingos deposit 100% of their excreta at the lake (Batanero et al., 2017). On the other hand, according to our estimates, gull excreta is the most important external P source to FP (Fig. S3).

Flamingos prefer deeper water and their numbers decline faster than gull numbers when water levels drop at the lake (Batanero et al., 2017). Gull guanotrophy effects are thus particularly likely at FP when winter water levels are low (e.g. winters 2014-2015, 2015-2016 and 2016-2017), since gull numbers remain high and nutrients further concentrate in the water column. We are not

aware of estimates of N and P inputs to the lake from runoff from the relatively small watershed and from the aquifer, so the overall relative contribution of gulls to the nutrient budget remains unclear, but it is likely to be a major fraction of the overall budget.

Ecosystem effects of guantrophy

Gull populations have previously been shown to cause guantrophy at roost sites, including drastic changes in ecosystem state such as a shift from clear water and high diversity into a turbid, low diversity state (Moss, 1994; Signa et al., 2015). Since P input from excreta is more rapidly bioavailable than P from runoff, which is mainly bound to the sediment, it is more likely to trigger rapid effects on algal growth and chlorophyll content (Winton & River, 2017).

Transportation of external nutrients into FP is a concern for managers as it affects the biodiversity of the lake (de-los-Ríos-Mérida et al., 2017). Eutrophication is considered responsible for a loss of diversity of aquatic plants at FP since the 1990s (Junta de Andalucía, 2005). The recent proliferation of the filamentous alga *Ulva flexuosa* in the lake is one indication of eutrophication (Conde-Álvarez et al., 2012). Guantrophy by gulls is likely to be one cause of these changes.

The biogeochemical effects of gull guano are likely to be strongly conditioned by the high salinity of the lake, which is likely to reduce methane production but increase phosphate release from the sediment (Clavero et al., 1990; Camacho et al., 2017). The water column in FP holds higher concentrations of N than of P, but nutrient dynamics are highly dependent on hydrology (García et al., 1997; Batanero et al., 2017). Batanero et al., (2017) reported a TN:TP ratio of 49:1 (range 25-85) in the dry year 2011-2012 and 52:1 (range 30-93) in the wet year 2010-2011. Therefore, P appears to limiting in the system based on the Redfield ratio 16:1 at which primary production is expected to switch from N-limitation to P-limitation (Redfield, 1958). On the other hand, P-rich guano from gulls and other waterbirds may contribute to microbial activity in the lake (Batanero et al., 2017). Moreover, P and uric acid (N) in excreta tends to accumulate in the sediment, partly delaying eutrophication effects until those nutrients are

released again due to disturbance or changes in water conditions (Dessborn et al., 2016).

Furthermore, reductions in water level in dry years and due to human activities (Rodríguez-Rodríguez et al., 2005) increase nutrient concentrations in the FP water column by 3-fold (Batanero et al., 2017). Ongoing climate change in Andalusia acts in synergy with nutrient loading and water extraction to further enhance their impacts (Espinar et al., 2015), and reductions in nutrient loading are required to ensure that Mediterranean wetlands such as FP maintain their resilience to climate change (Green et al., 2017).

Future studies

For studies such as ours based on movement and count data, acquiring more data on spatial and temporal variation in nutrient concentrations in excreta and on defecation rates is likely to be the best way to further improve estimates of nutrient inputs. Future work should also consider the implications of carbon inputs by gulls to lakes and reservoirs, which may reduce methane emissions from landfills by substituting anaerobic decomposition for aerobic respiration (Winton & River, 2017). Further distinction between behaviours within trips, for example using accelerometry, would be useful to understand in more detail how birds are using different foraging habitats within their home range. Finally, integrating movement analysis also allows the identification of source areas for contaminants, plastics, pathogens or alien species likely to be imported to lakes such as FP (Fig. S4). Gulls using landfills may transport harmful bacteria such as *Salmonella* or *E. coli* including strains resistant to antibiotics (Dolejska et al., 2009; Ahlstrom et al., 2018), as well as a range of contaminants (Belant, 1998).

Conclusions

Population increases of opportunistic, omnivorous birds such as gulls can pose threats to freshwater ecosystems. Fuente de Piedra is a good example of how, even when strictly protected, inland lakes can be impacted by inputs of nutrients and other matter imported by birds from outside the watershed. Ultimately, this

is a consequence of the dramatic changes in land use during the anthropocene, which are particularly well represented by landfills. Efforts are required to reduce nutrient inputs to freshwater ecosystems from gulls feeding at landfills, in line with the recommendations under the landfill directive (e.g. refuse reduction, and improved waste separation and recycling).

Interest in guantrophication processes in inland waters is long-standing, but ongoing developments in tracking technology allow guantrophy to be studied with greater precision. Advances in tracking methods and reductions in costs make it increasingly feasible to integrate studies of waterbird movements into studies of nutrient loading, and into management of freshwater habitats.

Acknowledgements

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Supplementary materials

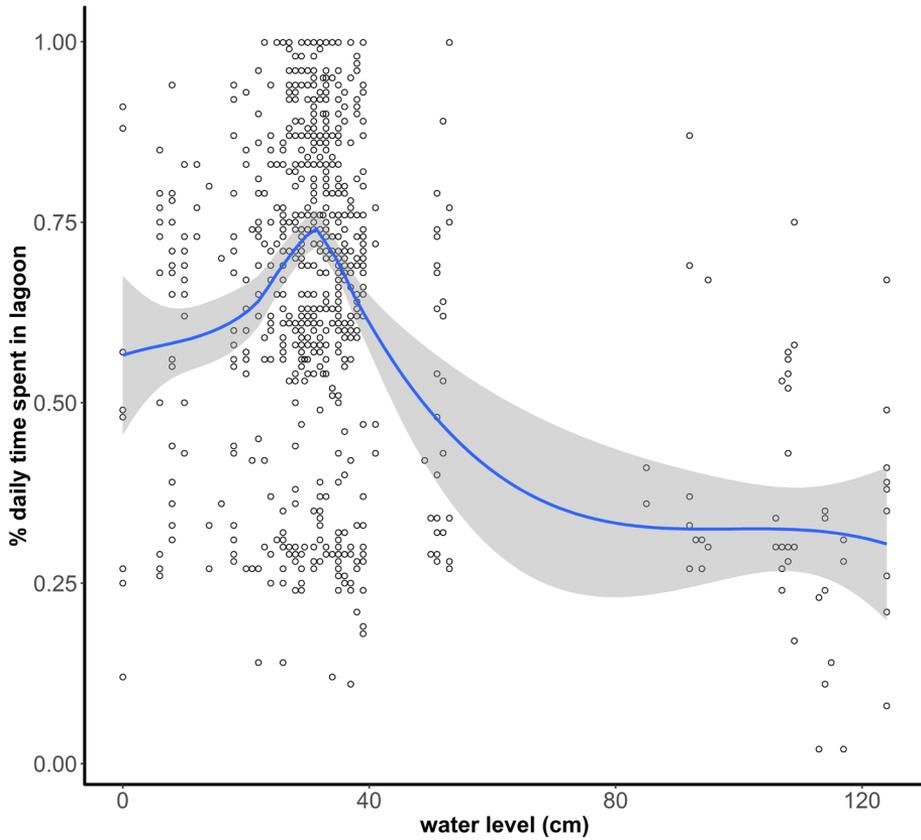


Figure S1. Relationship between the percentage of daily time spent in Fuente de Piedra and the water level in the lake. The trend line (with 95% CI) was fitted with the non-parametric LOESS function using a span value of 0.75.



Figure S2. Feathers shed by roosting gulls in Fuente de Piedra, an additional source of nutrient inputs not quantified in this study. Photo by Irene Paredes (January 2018).

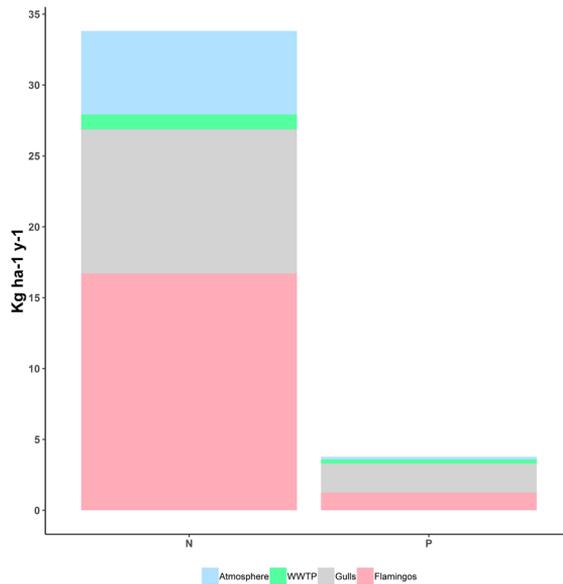


Figure S3. Estimated relative contribution ($\text{Kg ha}^{-1} \text{y}^{-1}$) of nutrient loading of gulls compared to other sources identified from the literature (flamingos, atmospheric inputs and Waste Water Treatment Plants (WWTP)). See discussion for more details.

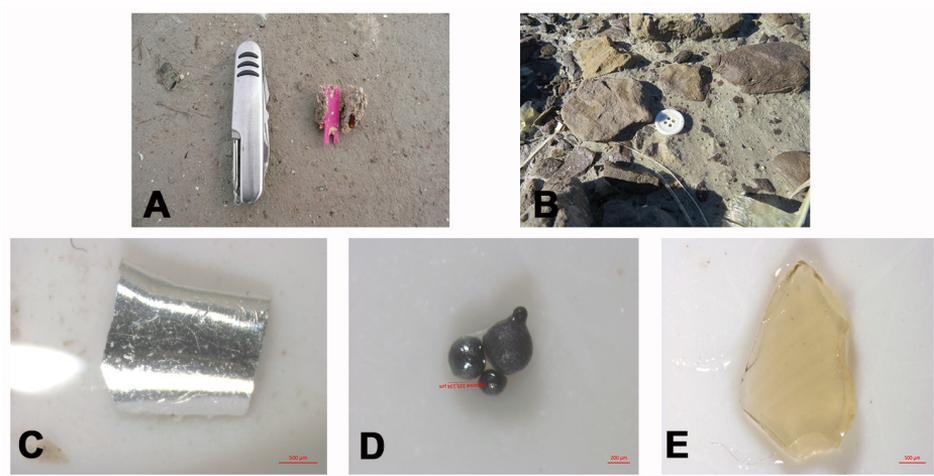
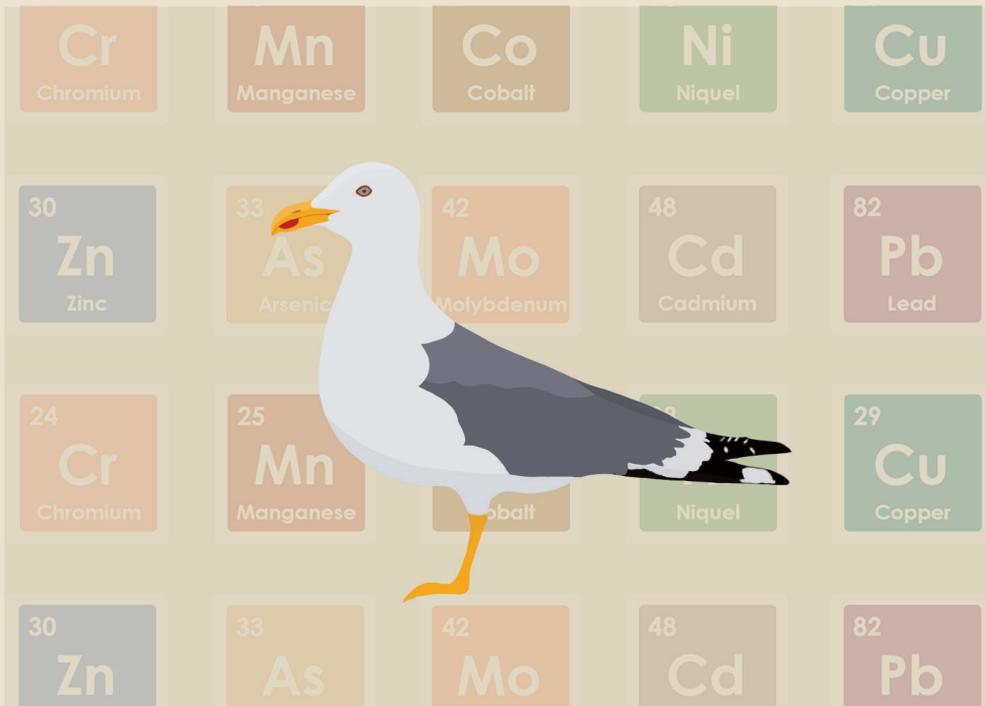


Figure S4. Examples of different waste types egested in pellets by LBBG in FP, and therefore imported to the lake from landfills: (A,B) different plastics, (C,E) different metals and (E) glass.

Chapter 5

Gulls as bioindicators and biovectors of heavy metal pollution



Martín-Vélez V., Hortas F., Taggart M.A., Green A.J., O'Hanlon N.J., Sánchez M.I. Gulls as bioindicators and biovectors of heavy metal pollution. In review in *Ecological Indicators*.

Abstract

We assessed the spatial variation in concentrations of ten heavy metals in faeces of the lesser black-backed gull (LBBG) *Larus fuscus* wintering at seven localities in SW Spain, to evaluate its potential as a bioindicator of environmental contamination.

We found high concentrations of metals in gull faeces, with several elements (As, Cu, Mo, Pb, Zn) locally exceeding (between 2 and 11 times) derived Lowest Effect Level (LEL) values. We also found strong spatial variation, related to the main pollution sources associated with the different sites. Faeces from Chipiona Port (Gulf of Cádiz) showed the highest levels of As; Cetina saltpans (Gulf of Cádiz) ranked first for Pb, Zn and Mo, which was consistent with historic mining and industrial pollution; Doñana ricefields showed the highest levels of Mn, a highly available element in flooded areas; while landfills ranked first for Cd, Co, Cr, Cu and Ni, potentially associated with electronic waste.

Furthermore, we demonstrate how such data can be used to quantify biovectoring of metals into specific localities, using LBBG movement ecology and census data. At Fuente de Piedra, an important protected inland lake, we show that metal inputs by LBBG have increased in recent years, and long-term deposition (e.g., of Pb) may impact aquatic communities and ecological processes in the lake. We review 35 existing studies of faecal metals in birds and conclude that this method is underutilized, given the need for more biovectoring studies.

Introduction

Anthropogenic activities (e.g., industrial processes, urban and agricultural practices) are increasingly contributing to environmental pollution worldwide (Baby et al., 2010; Vareda et al., 2019). Aquatic ecosystems are particularly vulnerable because pollutants not only cause direct impacts on biota, resulting in lethal or sub lethal effects, but also a variety of indirect perturbations through trophic cascades that can result in dramatic changes in food webs, ecosystem structure and nutrient fluxes (Fleeger et al., 2003; Baby et al., 2010). Among

the most prevalent and harmful contaminants in aquatic ecosystems are heavy metals (Deb & Fukushima, 1999). Many are highly toxic and persistent and can bioaccumulate and biomagnify through food webs (Goodyear & McNeill, 1999). Understanding how toxic metals enter and distribute within aquatic environments, and identifying potential bioindicators of contamination in the environment, are critical points in evaluating the risks heavy metals pose to the environment, wildlife and human health.

While pathways and entry routes of heavy metals into aquatic ecosystems via abiotic (physical) mechanisms are well characterized, the role of biological transport has been widely overlooked (Blais et al., 2007). Biovectors are often considered as negligible when looking at pollution transport pathways in a global context (see Kallenborn & Blais, 2015, for a recent review). However, there exists increasing evidence of pollution transport within and among ecosystems via biota (Michelutti et al., 2010), which can, in some cases, even exceed that mediated by abiotic pathways (Xie & Sun, 2008; Chu et al., 2019); for example, several studies have shown a relationship between contaminant distribution and migration of vertebrates at regional and inter-continental scales (Evenset et al., 2007; Michelutti et al., 2009; Kallenborn & Blais, 2015).

Despite this recent interest, there are important gaps in knowledge that make it difficult to evaluate the magnitude and widespread character of this phenomenon. For example, the main focus of research on biovectors is commonly in marine and terrestrial environments (Kallenborn & Blais, 2015), whereas inland waters have received little consideration. At the same time, such systems can be among the most diverse and threatened aquatic ecosystems and may be particularly susceptible to contaminants (whether by abiotic or biotic processes). In particular, contaminants are readily concentrated in closed-basin lakes without an outflow. Moreover, many studies focus on tissues and other structures (Chen & Hale, 2010), but studies of biotransport through faeces remain limited (Evenset et al., 2007; Desjardins et al., 2019). From an applied perspective, animal excreta can also be a valuable tool in biomonitoring of trace metals in the environment as they can reflect metal levels in blood, and sample collection is non-destructive, low-cost and relatively easy (Varsha, 2013; Berglund, 2018).

The role of waterbirds as both biovectors and bioindicators of metals can be investigated using their excreta (Michelutti et al., 2009; Martinez-Haro et al., 2011). They are important components of aquatic ecosystems, and are able to move between water bodies and across boundaries between terrestrial and aquatic systems (Green & Elmberg, 2014; Soininen et al., 2015; González-Bergonzoni et al., 2017), transporting and subsequently releasing contaminants into receiving ecosystems (Blais et al., 2007). Furthermore, because they are abundant, long-lived, widely distributed, and often sit at a high trophic level, they are excellent candidates for biomonitoring of environmental pollution (Green & Elmberg, 2014).

Gulls in particular are of significant interest because they feed opportunistically and are widely adapted to utilise a range of anthropogenic habitats (such as ports and landfills) whilst commonly returning to roost in otherwise comparably unpolluted waterbodies (Winton & River, 2017; **Chapter 4**). As a result of their highly gregarious behaviour, roosting and feeding in large flocks, they then have the potential to generate “hotspots” of contamination by incorporating contaminants from human-influenced feeding habitats and releasing these into their wetland roost sites (via their faeces). In Spain, gull guano has been shown to contribute to eutrophication in the Fuente de Piedra lake, a very important roosting site for gulls during the wintering period (**Chapter 4**). Given the ability of omnivorous gulls to ingest and potentially concentrate pollutants (as a result of their high trophic position and feeding habits; Ramos et al., 2013; Peterson et al., 2017), similar processes to those at play for nutrients may also operate with respect to other contaminants (Choy et al., 2010).

The lesser black-backed gull *Larus fuscus* (hereafter LBBG) is a long-distance migrant (Cramp & Simmons, 1983) whose population has experienced a progressive expansion throughout its range during the second half of the 20th century, and it is currently the second most abundant wintering species in Andalusian wetlands after the northern shoveler *Anas clypeata* (Junta de Andalucía). The success of this generalist species is widely attributed to its high adaptability and plasticity, which allows it to efficiently use human-modified

habitats (Gyimesi et al., 2015; **Chapters 1 and 4**). Thus, it represents a good model system to study biotransport of contaminants during the winter. As far as we know, there are no studies evaluating the role of gulls as biovectors of heavy metals in wetland ecosystems. Furthermore, there are also no studies of heavy metals in LBBG faeces and very few on other gull species. Given the abundance and movements of gulls, such data would be important to monitor potential changes in the environment, particularly in protected wetlands or agricultural systems relevant for human health.

The aim of this study was to investigate the potential for LBBG to act as biovectors and bioindicators of metal contamination (including heavy metals and the metalloid Arsenic) using their excreta. We provided data on the concentrations of 10 elements (including both essential and toxic metals) in faeces collected from sites in south-west Spain with different degrees of anthropogenic influence and used regularly as roosting and feeding habitats during wintering seasons.

The specific objectives were:

(1) To study spatial differences in the content of metals in gull samples from different foraging and roosting sites, including protected wetlands, landfills and important areas for human food production such as fishing ports, ricefields and saltpans, during the wintering season. We anticipated that heavy metal concentrations in gull faeces should reflect key pollution sources associated with these different environments.

(2) To quantify heavy metal biotransport to one of Spain's most important natural lakes, Fuente de Piedra, based on faecal analysis, censuses and movement ecology.

(3) To perform a review of studies reporting metals in bird faeces in order to compare them with our results for LBBGs, and to identify potential knowledge gaps and challenges.

Materials and methods

Study area

This study was carried out across seven sites with different degrees of human influence, used for roosting and feeding by LBBG (see Fig. 1). Foraging sites provide information on dietary exposure to heavy metals (Kim et al., 2009) and act as ‘sources’ of heavy metals that may ultimately be deposited in wetland roosting sites, which may then act as heavy metal ‘sinks’ (Simpson et al., 1983).

These sites were:

- (1) Doñana Ricefields in the Guadalquivir marshes (Seville, 37,000 ha), the largest area devoted to rice production in Spain, accounting for up to 42% of total national crop. This area is located in one of the most important wetlands for migratory waterbirds in the Western Palearctic (Rendón et al., 2008). Doñana ricefields provide important habitat for LBBG and other waterbirds (Toral & Figuerola, 2010) both for feeding (e.g., on the alien crayfish *Procambarus clarkii*) and roosting (Lovas-Kiss et al., 2018a; **Chapter 1**).

- (2) Fuente de Piedra lake (Málaga, 1,350 ha) is the largest natural shallow lake in Andalusia, protected at regional (Natural Reserve), European (Special Protection Area) and international (Ramsar site) levels. It hosts one of the largest flamingo nesting colonies in the western Mediterranean (Bechet et al., 2012) and is a roosting area for over 20,000 LBBG in winter (**Chapter 1**). These LBBG feed mostly at landfills beyond the lake catchment area, and their excreta are a major cause of lake eutrophication in winter (**Chapter 4**).

- (3) Chipiona Port (2,605 m²), in the southern part of the Gulf of Cádiz, is an important feeding area for LBBG during the non-breeding season (Ramírez et al., 2015). Gulls benefit from high marine productivity nearby (LaFuente & Ruiz, 2007), which commonly peaks in late winter. In this period, gulls concentrate close to the port to feed on fishery discards (Bartumeus et al., 2010; Ramírez et al., 2015).

- (4) Cetina saltpan complex (also in the Gulf of Cadiz; 1,100 ha), created in 2014, one of the biggest saltpan complexes in Spain, and one of the most important in the area for salt production. It is listed as an Important Bird Area (IBA 251) (Infante et al., 2011), being an important unprotected feeding habitat for many species of waterbirds (Masero & Pérez-Hurtado, 2001), but LBBG use it mainly as a roosting site.

- (5, 6, 7) Landfill sites (two in Cádiz (VerinSur and Miramundo) and one in Córdoba). These landfills are used by large numbers of LBBG for feeding and are known to be connected with important roosting areas for LBBG (**Chapters 1 and 4**). These landfills receive waste from surrounding urban areas and contain elevated levels of heavy metals (de-la-Casa-Resino et al., 2014; Cabo et al., 2012).

Sample collection

All seven sites were visited between November 2017 and January 2018. Thirty faecal samples were collected at each site (n= 210). Only fresh faeces (i.e., visibly 'wet') were collected, after detecting monospecific LBBG flocks. Samples were taken from points separated by at least one meter, in order to increase the likelihood that they were from different individuals. Any sign of soil surface contamination was carefully removed before individually storing the samples in labelled zip-lock bags. Samples were preserved in the freezer (at -20°C) and later dried at 60°C (for 24 hours) prior to sample digestion for heavy metal analysis.

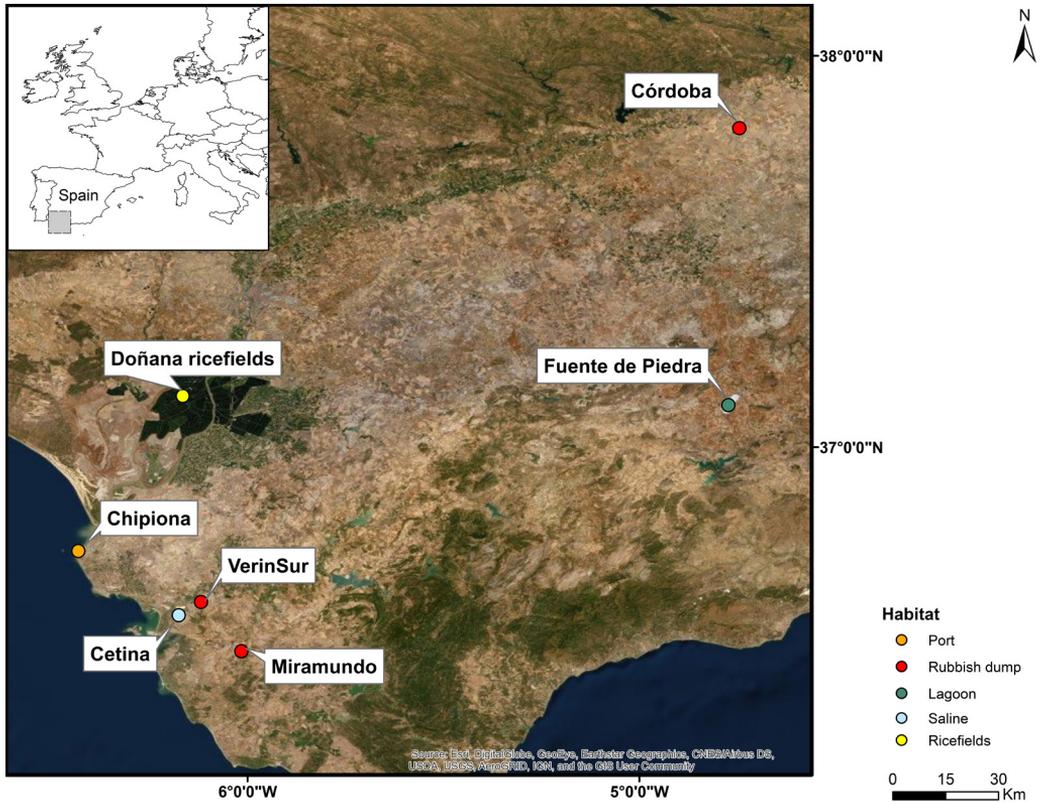


Figure 1. Study area showing the seven locations where LBBG faecal samples were collected in 2017-2018. Fuente de Piedra lake and Cetina salt pan are important roosting sites whereas Córdoba, VerinSur and Miramundo are landfills and foraging areas. The Donana ricefields are used for both foraging and roosting.

Sample digestion and ICP-OES analysis

For sample digestions, trace metal grade nitric acid (>67%) and hydrogen peroxide (>30%; Fisher Scientific) were used. Mixed standards for ICP-OES calibration and analysis were made using dilutions from certified 1000ppm stock solutions (Sigma-Aldrich) of each element of interest: arsenic (As), cadmium (Cd), cobalt (Co), chromium (Cr), copper (Cu), manganese (Mn), molybdenum (Mo), nickel (Ni), lead (Pb) and zinc (Zn). Certified Reference Materials (CRMs) were used to assess recovery of elements during the digestion and analytical process. The CRM's used were 'Lobster Hepatopancreas' (TORT-2; NRC) and 'Bushes, Branches and Leaves' (DC73348; NCS). The concentrations of each element in these CRMs along with their associated uncertainties are displayed in Table S1. All samples, CRMs and blanks were digested using the same procedure. Approximately 0.3-0.4g of sample was accurately weighed into a disposable sample weigh-boat before being added to a 50ml PTFE microwave digestion vessel. This was repeated to make up a 24-vessel digestion carousel which consisted of 21 samples, two CRMs and one blank (made up using 0.35ml of Milli-Q water, instead of sample). To each digestion tube 3.5ml of trace metal grade concentrated nitric acid was added and vessels were left overnight to pre-digest (cap placed loosely on top to allow fumes to escape). Each vessel then had 1ml of trace metal grade hydrogen peroxide and 1ml of Milli-Q water added the next day. The 24 vessel carousel was then placed into a microwave digestion reaction system (Anton Paar, Multiwave PRO), set to a program which ramped up to 110°C over 20 minutes, held for 15 minutes, ramped to 180°C over 15 minutes, held for 30 minutes, before finally cooling to 55°C using the maximum cooling fan setting (giving a total digestion time of approximately 1 hour and 40 minutes per 24 vessel carousel). Digestion vessels were then allowed to cool to room temperature before caps were carefully removed to allow the vessel pressure to release gradually. The digest liquid was then poured into a 15ml sample tube. The reaction vessel was then further rinsed with Milli-Q (three times), adding each rinse to the sample tube, before finally topping up to the 15ml mark with Milli-Q water. Digest vessels were then thoroughly washed with Type II deionised water (twice) and then placed in a wash bath made up with 3% DECON® for 24 hours. They were then rinsed twice with Milli-Q and placed in

an acid bath (5% nitric acid) for 24 hours before washing again with Milli-Q (3 times) and drying overnight. The above was then repeated until all samples were digested.

ICP-OES analysis was performed on a Varian 720-OES (Agilent) instrument. All standards were prepared in diluted trace metal grade nitric acid to matrix-match the acidity of the primary sample digests. To account for instrumental drift during analytical runs, a re-slope was undertaken every 10 samples using an intermediate standard (alongside a blank). All measurements were performed by analysing three 'potential' wavelengths for each element. Wavelengths used for the final data were selected based on CRM recovery as well as sensitivity (i.e., signal intensity) and the final Limit of Detection (LOD).

Statistical analyses

For statistical analysis metal values below the LOD were replaced by $0.5 \times \text{LOD}$. We used the seven sites sampled from November 2017 to January 2018 to perform principal component analysis (PCA) in order to identify associations between elements, and differences in element profiles between the seven locations. Data were log-transformed to reduce the influence of outliers and normalize their distribution. Differences between locations were tested using one-way ANOVAs. If differences were significant, we carried out post-hoc Tukey tests. All analyses were performed in R (v.3.4.1) with packages *multcomp* (Hothorn et al., 2017) and *factoextra* (Kassambara & Mundt, 2017). To evaluate the environmental/toxicological significance of the heavy metal levels measured in our samples we compared our data with several widely used SQGs (sediment quality guidelines) (Persaud et al., 1993; Deckere et al., 2011; Kabata-Pendias & Mukherjee, 2007). These were based on consensus values taking into account ecotoxicological values (Threshold Effect Levels (TEL): the concentration below which adverse biological effects are expected to occur rarely) and ecological values (Lowest Effect Levels (LEL): level of sediment contamination that can be tolerated by most benthic organisms) for freshwater ecosystems (Table 1 in results).

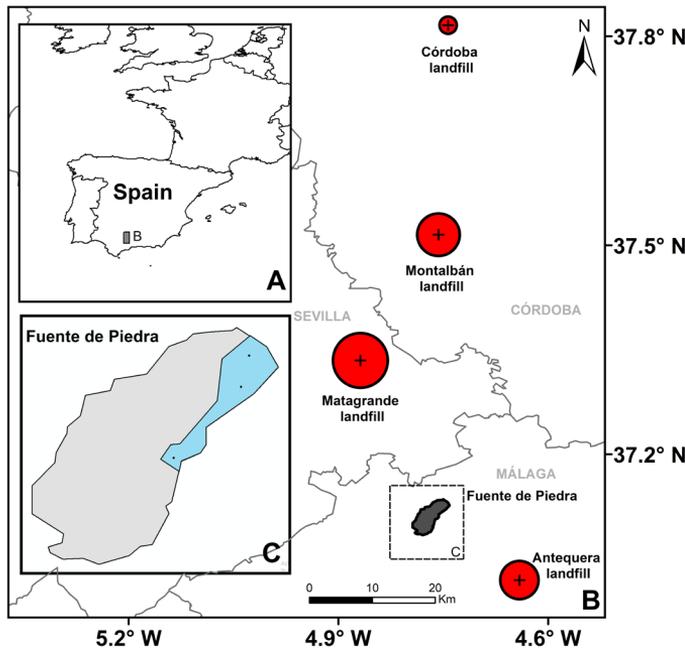


Figure 2. a) Location of Fuente de Piedra lake in the Iberian Peninsula. (b) Four landfills used as foraging sites by gulls making daily movements from Fuente de Piedra. The size of the circle is proportional to the total cumulative time gulls spent in each site. (c) Dots show the location of the three main roosting sites of gulls within Fuente de Piedra. Grey area show the whole area basin of the lake, whereas blue area the roosting sites for metal quantifications. Figure adapted from **Chapter 4**.

Estimations of heavy metal biotransport

We estimated Heavy Metal Load (HML) to Fuente de Piedra Lake (the main midwinter roosting site for LBBG in Andalusia) via LBBG faeces for seven winters (from 2010 to 2017). We adapted a nutrient quantification methodology used in **Chapter 4** to determine heavy metal transport to the lake. We provided estimations in g ha^{-1} related to the area that the specific roosting sites cover within the Lake (calculated in ArcMap based on satellite maps), as well as relative

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to the whole lake surface (Fig. 2). Roosting sites were identified after **Chapter 4**, which is also the source of our data inputs for gull counts and gull movements to and from landfills used for feeding.

We estimated each element load as follows:

$$\text{HML} = \text{TS} * \text{ER} * \text{ND} * \text{MN} * \text{EC}_{\text{faeces}}$$

Where TS = Time Spent, i.e., the average roosting time per year, for every year (from **Chapter 4**).

ER = Excretion Rate per individual per day (g day^{-1}), which was considered as a fixed parameter (21.06 g day^{-1}), calculated using equation 3 from the Hahn et al. (2007) model for nutrient transport by carnivorous birds:

$$\text{ER} = \alpha \times \text{DER} / (\text{E} \times \text{AM})$$

Wherein α = the intake to excretion ratio of 0.395 (Dobrowolski et al., 1993; Nixon & Oviatt, 1973). DER = daily energy requirement: 968.66 kJ (Nagy et al., 1999), assuming LBBG have a mean body mass of 792 g (Hahn et al., 2007). E = food energy content: 23.9 kJ g^{-1} , based on a landfill diet (Winton & River, 2017). AM = the metabolizable energy coefficient: 0.76 (Karasov, 1990).

Further, ND = Number of Days per winter (180 days, from September to February); while MN = Daily Mean Number of individuals (in 2018) of LBBG present, calculated from census data (provided by Junta de Andalucía). EC = the average Element Content measured in micrograms per gram of dry faeces.

Review of studies reporting metals in bird faeces

In order to compare our data with existing studies and to identify general gaps in knowledge, we undertook a systematic review of papers that included data for heavy metals in faeces (for any kind of bird). We searched in Google Scholar using the following combinations of key words: “excrement” OR “excreta” OR

“faecal” OR “fecal” OR “feces” OR “faeces” AND “heavy metal” AND “bird”. For each study, we extracted the average heavy metal concentration between all the locations included in the study. When several studies provided information regarding the same species, we reported the average and range of values of all the studies that belonged to the same bird species. We also recorded habitat type, country, and classified each bird according to its taxonomic group (e.g., duck, gull, passerine).

Results

Analysis of heavy metals in gull faeces

Principal component analysis reduced the complexity of our data from ten elements to two principal components that cumulatively explained ~78.2% of the variation in the data (62.3% for PC1, 15.9% for PC2 and 9.3% for PC3; Fig. 3). Moreover, elements were associated with two clear groupings: with PC1 reflecting changes in Co, Cu, Mo, As, Pb, Zn and a different signal for the Cetina salt pan in comparison to other locations; PC2 reflected an association with Mn, Cr and Ni (Fig. 3b). In descending order, Co, Cu and Mo (essential heavy metals) were the most contributing elements to PC1, whereas Mn, Ni and Cr contributed most to PC2 (Fig. S1). Cd was not related with either of the two main principal components, but instead to PC3 (at 94%; Fig. S1).

Overall, all the elements significantly differed between locations, (Fig. 4; Table S2). Zn was least variable, showing high values in most of the sites (four of them exceeded the LEL values and the TEL value for Cetina). Cu was also high, exceeding the LEL and TEL in all sites (Table 1). Faecal samples collected at Chipiona port (Gulf of Cádiz) had the highest level of As, surpassing the LEL value (Table 1, Fig. 4); however, it showed the lowest concentrations of Cd, Co, Cr, Cu, Mn, Ni and Pb. Faeces from the Cetina salt pans (Gulf of Cádiz) ranked first for Pb, Zn and Mo, with overall high values for all elements (four of them exceeded LEL values, with Mo exceeding the LEL value by more than 11 times). Samples collected at landfills ranked first for Cd, Co, Cr, Cu and Ni (Table 1),

high levels for most of the remaining elements (except for Zn, where the lowest concentrations occurred) (Table 1). Fuente de Piedra Lake showed similar values of heavy metals to the landfills, although it had the lowest values for As, and significantly lower Cr in comparison to the landfills (Fig. 4).

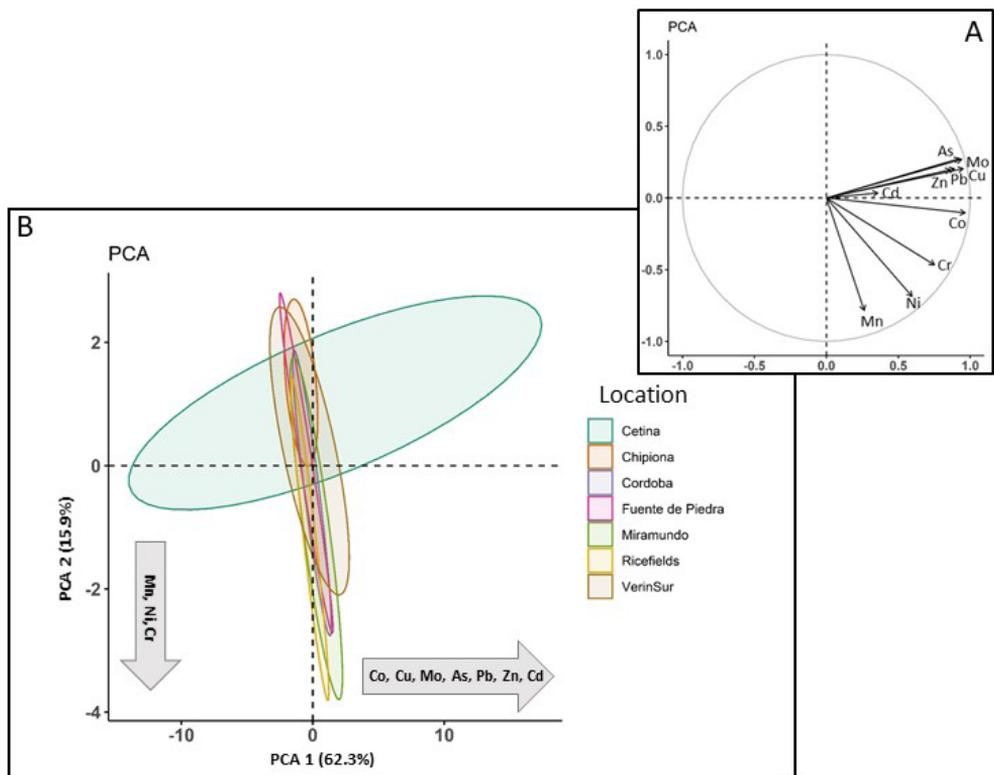


Figure 3. (A) Principal component analysis (PCA) to illustrate the associations between elements and the degree of contribution of each element to the two main dimensions. (B) Two dimensional PCA space with 95% confidence interval ellipses for faeces element profiles for each location to illustrate differences in element profiles. Arrows show the direction of an increasing gradient of each element along the axis where they contribute the most (the order of the elements reflects decreasing contributions to the axis). PC3 is not shown but is highly related to Cd (see text).

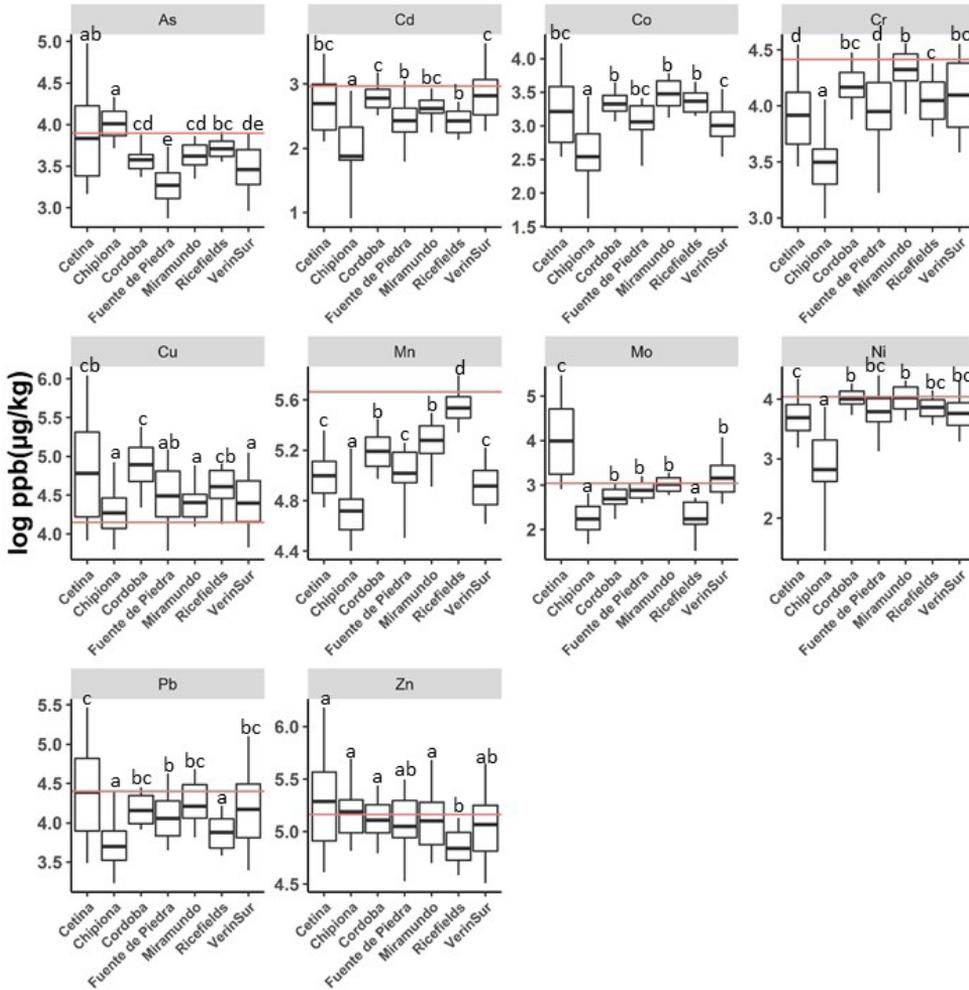


Figure 4. Comparison between seven localities for each of the ten elements. Boxplots show the geometric mean of log-transformed data with the 25 and 75th quartiles, whereas whiskers show the 95% CI. Red line shows the consensus values for Lowest Effect Level (LEL) and Threshold Effect Levels (TEL). Significant differences ($p < 0.05$) between locations in the concentrations of the different elements are represented by different letters above the bars, based on a Tukey post-hoc test.

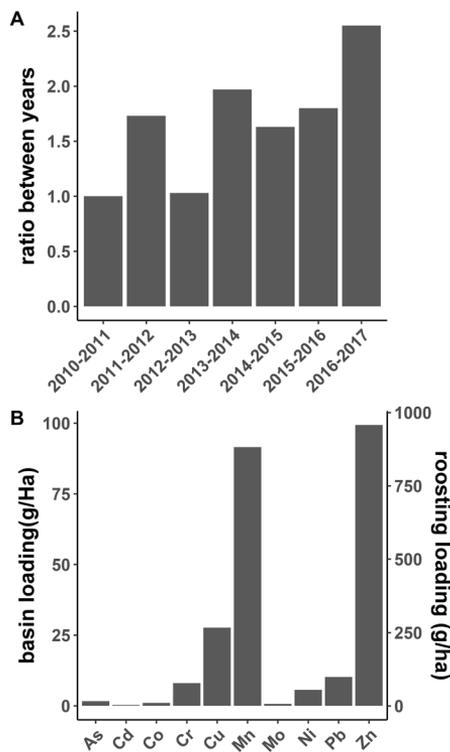
Table 1. Summary table of geometric mean (and 95% CI; in $\mu\text{g/g}$ of faeces) for the ten elements analysed (As, Cd, Co, Cr, Cu, Mn, Mo, Ni, Pb and Zn) in faecal samples ($N = 30$) among seven locations in SW Spain, with samples collected in November 2017 (in ricefields), December 2017 (Cordoba, VerinSur and Miramundo landfill) and January 2018 (Fuente de Piedra, Chipiona and Cetina). ‘Thresholds’ based on consensus values using both ecotoxicological values (Threshold Effect Levels (TEL)) and ecological values (Lowest Effect Levels (LEL)) were reported when available in literature. Levels above the thresholds were marked with 1 (for LEL) and 2 (for TEL).

Element	Threshold	Chipiona port	Cordoba landfill	Fuente de Piedra lake	Miramundo landfill	Cetina salt pan	Doñana ricefields	Verinsur landfill	
As	LEL	7.9	10.31	3.8	1.9	4.2	7.6	5.2	3
	TEL	-	(8.6-12.3)	(3.3-4.3)	(1.5-2.4)	(3.7-4.9)	(4.4-13.0)	(4.7-5.7)	(2.3-3.9)
Cd	LEL	0.71	0.1	0.6	0.3	0.4	0.5	0.3	0.7
	TEL	1.2	(0.1-0.6)	(0.5-0.7)	(0.2-0.4)	(0.4-0.5)	(0.4-0.8)	(0.2-0.3)	(0.5-1.1)
Co	LEL	-	0.4	2.1	1.2	3	1.8	2.4	1.1
	TEL	-	(0.3-0.6)	(1.8-2.5)	(0.9-1.6)	(2.5-3.7)	(1.1-3.0)	(2.0-2.8)	(0.8-1.4)
Cr	LEL	25	3.3	14.8	9.3	21.2	8.6	11.3	12.8
	TEL	26	(2.5-4.2)	(12.6-17.3)	(6.6-13.0)	(17.9-25.2)	(6.1-12.1)	(9.4-13.6)	(9.6-17.2)
Cu	LEL	13	19.2 ^{1,2}	79.5 ^{1,2}	32 ^{1,2}	26 ^{1,2}	67.7 ^{1,2}	41.1 ^{1,2}	26.2 ^{1,2}
	TEL	16	(13.7-27.0)	(60.4-104.7)	(22.7-45.1)	(20.2-33.5)	(35.8-127.7)	(32.7-51.7)	(18.5-37.1)
Mn	LEL	-	52.8	155.8	105.9	191.6	100	345.2	83.4
	TEL	460	(43.3-64.3)	(134.6-180.3)	(85.6-130.9)	(161.6-227.3)	(83.8-119.4)	(304.5-391.3)	(70.4-98.9)
Mo	LEL	1.1	0.2	0.5	0.8	1.11	12.21	0.2	1.61
	TEL	-	(0.1-0.3)	(0.4-0.7)	(0.6-0.9)	(0.8-1.2)	(5.7-26.0)	(0.1-0.3)	(1.0-2.4)
Ni	LEL	15	0.8	10.12	6.5	10.72	5.1	7.3	5.9
	TEL	7.5	(0.5-1.4)	(8.8-11.7)	(4.8-8.8)	(8.8-13.0)	(3.8-6.9)	(6.2-8.6)	(4.6-7.8)
Pb	LEL	19	5.2	14.5	11.8	16.8	27.61	7.6	16
	TEL	31	(3.7-7.2)	(12.1-17.3)	(8.6-16.2)	(12.6-22.3)	(14.8-51.5)	(6.3-9.4)	(10.5-25.5)
Zn	LEL	129	157.11	129.91	114.9	129.61	203.7 ^{1,2}	69.6	120.3
	TEL	163	(122.6-201.2)	(109.1-154.7)	(88.6-148.9)	(96.0-174.8)	(130.7-317.5)	(58.4-83.0)	(86.1-168.3)

Heavy metal input into the Fuente de Piedra lake

Based on the average heavy metal content in faeces, the percentage of time LBBG spent at Fuente de Piedra lake per winter, and the corrected census data from **Chapter 4**, we determined the heavy metal loading caused by LBBG faeces into Fuente de Piedra lake over seven winters (Fig. 5). Heavy metal load strongly varied between years, but overall, there was an increasing trend, primarily because of increased gull numbers in recent years and an increase in the time spent roosting. Our estimations of heavy metal load via gull faeces assigned maximum values (in g ha^{-1}) to the essential elements Mn and Zn and minimum values to Cd and Mo (Fig. 5b). By way of example, in terms of the toxic

heavy metal Pb, the total amount deposited over the last year (2016-2017) was estimated to be 76.88 g ha⁻¹ to the roosting sites and 10.18g ha⁻¹ to the whole lake basin (solely due to LBBG faecal inputs), and 13.76 Kg in total mass (see Fig. 2 for differences between roosting site and whole basin). Alternatively, using the ‘average’ amount over this 7-year period, just over 5Kg ha⁻¹ per century were deposited in roosting sites and a total of 902 Kg per century.



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Figure 5. A) Index of heavy metal loading by LBBG in the roosting location at Fuente de Piedra lake (South Spain) between seven study years, taking the first winter/year 2010-2011 as reference. B) Estimated element inputs (in grams per hectare) by LBBG's over the last winter (from 2016- 2017) for both roosting sites and the whole lake basin.

A total of 35 studies were identified which included heavy metal analyses of avian faeces (Table 2). These considered 45 different bird species and 16 taxonomic groups. Fourteen countries from four continents and ten different habitats were identified where the studies were carried out. Pb was the most reported element (90% of studies) followed by Cu (in 84%). Mo has not been reported before in any of the studies identified. The highest values of As and Zn were reported for the common whitethroat *Sylvia communis* from agricultural land in Poland (Table 2). Studies related to Indian cities showed the highest levels of Cd, Cr and Pb, in red-wattled lapwing *Vanellus indicus*, and of Mn in the Eurasian collared dove *Streptopelia decaocto* (Table 2). High levels of Co were found in great tit *Parus major* along an environmental gradient in different European countries. The Humboldt penguin *Spheniscus humboldti* showed the highest levels of Cu, in the Antarctic. The highest levels of Ni were reported in chickens *Gallus gallus domesticus*, in a city in Venezuela.

Among gulls, ours is the most complete study in terms of variety of elements and reports the highest (or only) values for As, Co, Cr, Mn and Mo (Table 2). The highest values for Cd, Pb, and Zn in gulls were reported in Spain for yellow-legged gull *Larus cachinnans*; whereas Ni and Cu were most elevated in Saunders's gull *Larus saundersi* in China (Table 2).

Table 2. Summary table of studies on heavy metals in bird faeces. Element concentration given in µg/g (mean and range between brackets provided when various studies involved the same species). Bold numbers reflect the rank for each element (whereby 1 has the highest ranked mean concentration).

	Habitat	Functional group	Common name	Species	Location	As
1	Wetland	Anatidae	Mallard	<i>Anas platyrhynchos</i>	Spain	-
2	Wetland	Anatidae	Greylag geese	<i>Anser anser</i>	Spain	-
3	Agricultural land	Apodidae	Common swift	<i>Apus apus</i>	Poland	24. 0.3
4	Agricultural land	Ardeidae	Great Blue Heron	<i>Ardea herodias</i>	EEUU	-
5	City	Ardeidae	Cattle egret	<i>Bubulcus ibis</i>	India	12. 2.3 (2.1-2.4)
6	Swamp, landfill	Ardeidae	Little Blue heron	<i>Egretta caerulea</i>	EEUU	-
7	City	Charadriidae	Red-wattled lapwing	<i>Vanellus indicus</i>	India	4. 7.5
8	Wetland	Crane	Common crane	<i>Grus grus</i>	China	17. 0.7
9	Wetland	Crane	Demoiselle crane	<i>Grus virgo</i>	China	21. 0.5
10	Wetland	Crane	Red-crowned crane	<i>Grus japonensis</i>	China	20. 0.5
11	Wetland	Crane	White-naped crane	<i>Grus vipio</i>	China	19. 0.6
12	City	Dove	Rock dove	<i>Columba livia</i>	India	23. 0.3 (0.2-0.4)
13	City	Dove	Eurasian collared dove	<i>Streptopelia decaocto</i>	India	11. 3.5 (0.6-6.4)
14	Port	Gull	Yellow-legged gull	<i>Larus cachinnans</i>	Spain	-
15	Wetland, Landfill, Port	Gull	Lesser Black-backed gull	<i>Larus fuscus</i>	Spain	6. 5.1 (1.5-13.0)
16	Wetland	Gull	Saunders's gull	<i>Larus saundersi</i>	China	15. 1.1
17	Island	Gull	Sooty tern	<i>Onychoprion fuscatus</i>	EEUU	-
18	Arctic	Gull	glaucous gull	<i>Larus hyperboreus</i>	Arctic	-
19	Antartic	Gull	Black headed gull	<i>Larus argentatus</i>	Antartic	-
20	City	Other	Chicken	<i>Gallus gallus domesticus</i>	Venezuela	-
21	City	Parakeet	Rose-ringed parakeet	<i>Psittacula krameri</i>	India	14. 1.6
22	City	Passerine	Rook	<i>Corvus frugilegus</i>	Russia	-
23	City	Passerine	House crow	<i>Corvus splendens</i>	India	16. 0.8 (0.7-0.9)
24	City	Passerine	Common myna	<i>Acridotheres tristis</i>	India	8. 4.9 (0.8-8.9)
25	River shed	Passerine	American dipper	<i>Cinclus mexicanus</i>	Canada	10. 4.1
26	Smelter	Passerine	Pied flycatcher	<i>Ficedula hypoleuca</i>	Finland, Sweden	9. 4.8(0.02-10.6)
27	Smelter, Factory	Passerine	Eurasian blue tit	<i>Parus caeruleus</i>	Belgium, Finland	5. 6.0 (0.9-16.0)
28	Smelter, Forest, Factory	Passerine	Great tit	<i>Parus major</i>	Belgium, Finland, Portugal	3. 7.6*(0.1-159)
29						
30	City	Passerine	Common babbler	<i>Turdoides caudatus</i>	India	7. 4.9
31	Agricultural land	Passerine	common whitethroat	<i>Sylvia communis</i>	Poland	1. 35.3
32	Antartic	Penguin	Gentoo penguin	<i>Pygoscelis papua</i>	Antartic	22. 0.4
33	Antartic	Penguin	Humboldt penguin	<i>Spheniscus humboldti</i>	Antartic	2. 7.9
34	Antartic	Penguin	Adelie Penguin	<i>Pygoscelis adeliae</i>	Antartic	-
35	Antartic	Penguin	Emperor penguin	<i>Aptenodytes forsteri</i>	Antartic	-
36	Antartic	Penguin	Chinstrap penguin	<i>Pygoscelis antarctica</i>	Antartic	-
37	Antartic	Penguin	Gentoo penguin	<i>Pygoscelis papua ellsworthii</i>	Antartic	6. 5.1
38	Wetland	Rallidae	Eurasian coot	<i>Fulica atra</i>	Spain	-
39	Wetland	Rallidae	Western swamphen	<i>Porphyrion porphyrio</i>	Spain	-
40	Agricultural land	Raptor	Peregrine falcon	<i>Falco peregrinus</i>	Sweden	-
41	Agricultural land, river	Raptor	spotted owl	<i>Athene brama</i>	India	13. 2.1 (0.8-3.4)
42	River shed	Raptor	Bald Eagle	<i>Haliaeetus leucocephalus</i>	EEUU	-
43	Antartic	Sea bird	Giant petrel	<i>Macronectes Giganteu</i>	Antartic	-
44	Island	Sea bird	Booby	<i>Sula sula</i>	China	-
45	Wetland	Stork	Oriental white stork	<i>Ciconia boyciana</i>	China	18. 0.7
46	City	Vulture	Black vulture	<i>Coragyps atratus</i>	Venezuela	-

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	Cd	Co	Cr	Cu	Mn	Mo
1	-	-	-	-	-	-
2	-	-	-	-	-	-
3	4.8.4	3.1.1	-	31.0.9	15.0.3	-
4	32.0.2 (0.1-0.5)	4.0.6 (0.3-0.8)	21.1.3 (0.7-3.3)	-	-	-
5	17.2.0 (1.9-2.1)	-	6.23.1 (22.7-23.5)	22.17.5 (16.3-18.7)	2.1838.3 (242.3-3434.3)	-
6	31.0.2 (0.1-0.3)	-	-	-	-	-
7	1.48.8	-	1.86.3	8.87.4	3.760.4	-
8	29.0.3	-	12.6.6	26.8.6	-	-
9	28.0.4	-	19.3.2	23.17.0	-	-
10	23.0.7	-	13.5.9	24.16.9	-	-
11	24.0.7	-	17.3.8	25.11.8	-	-
12	30.0.2 (0.2-0.3)	-	15.5.1 (3.42-6.8)	9.75.9 (32.4-119.5)	10.138.0 (128.7-147.3)	-
13	20.1.7 (0.7-2.7)	-	4.32.4 (9.4-55.4)	12.53.1 (20.7-85.5)	1.2152.1 (625.1-3679)	-
14	7.5.8	-	8.9.8	10.60.1	-	-
15	27.0.4 (0.2-1.1)	2.1.7 (0.3-3.7)	7.11.6 (2.5-25.2)	15.41.7 (13.7-127.9)	9.147.8 (43.2-391.3)	1.2.3 (0.1-26.0)
16	14.3.1	-	11.6.9	7.98.4	-	-
17	13.3.6	-	-	-	-	-
18	-	-	-	-	-	-
19	-	-	-	-	-	-
20	-	-	-	17.38.9	-	-
21	15.2.1	-	5.29.9	16.40.2	6.323.7	-
22	-	5.0.04 (0.03-0.04)	-	30.1.47(0.49-2.45)	14.2.3 (2.2-2.4)	-
23	18.1.9 (0.1-3.8)	-	16.4.9 (2.7-7.1)	19.27.0 (9.9-44.1)	9.133.7 (45.6-221.8)	-
24	8.5.3 (0.6-10.0)	-	2.61.0 (12.9-109.2)	14.46.6 (26.2-67.0)	4.620.3 (164.4-1075.8)	-
25	9.4.9	-	-	13.50.6	8.259.4	-
26	10.3.7 (1.3-5.4)	-	-	2.158.3 (3.3-269.4)	-	-
27	-	-	-	-	-	-
28	11.3.7 (1.3-9.4)	-	-	5.124.5 (37.5-20)	-	-
29	-	-	-	-	-	-
30	12.3.6*(0.9-16.8)	1.4.9 (2.9-7.8)	18.3.2 (2-4.7)	4.145.3 (10.7-47)	7.273.5 (130-523)	-
31	-	-	-	-	-	-
32	16.2.1	-	3.33.8	3.150.8	5.401.4	-
33	3.9.9	-	10.7.1	11.55.7	-	-
34	21.1.3	-	-	-	-	-
35	19.1.7	-	-	1.199.7	-	-
36	6.6.3	-	-	-	-	-
37	-	-	-	-	-	-
38	-	-	-	-	-	-
39	22.1	-	20.2.1	6.104	13.12.3	-
40	-	-	-	-	-	-
41	-	-	-	-	-	-
42	25.0.6	-	-	28.3	-	-
43	26.0.6 (0.4-0.8)	-	9.7.4 (3.5-12.1)	18.31.1 (12.7-54)	11.103.1*(45.8-165.2)	-
44	-	-	-	29.2.9	12.43.2	-
45	-	-	-	-	-	-
46	5.6.3	-	-	20.21.1	-	-
47	23.0.7	-	14.5.6	27.7.1	-	-
48	2.13.9	-	-	21.20.3	-	-

*continue in next page

	Ni	Pb	Zn	Reference
1	-	21. 6.1 (2.2-9.6)	-	Martinez-Haro et al., 2011
2	-	24. 5.0 (2.2-15.0)	-	Martinez-Haro et al., 2013
3	-	40. 0.5	28. 5.8	Orlowski et al., 2015
4	-	31. 2.5 (0.4-6.0)	-	Fitzer et al., 1995
5	11. 11.7 (10.8-12.5)	18. 7.8 (7.2-8.3)	20. 132.8 (128.1-137.5)	Kaur & Dhanju, 2013; Kler et al., 2014
6	-	32. 1.5 (1.5-1.6)	-	Spahn & Sherry, 1999
7	2. 43.4	1. 864.0	2. 474.0	Kaur & Dhanju, 2013
8	13. 8.5	12. 14.6	25. 58.3	Fu et al., 2013
9	15. 7.9	19. 7.0	24. 107.9	Fu et al., 2013
10	16. 7.0	14. 13.2	19. 142.4	Fu et al., 2013
11	21. 6.1	20. 6.4	23. 116.8	Fu et al., 2013
12	14. 8.2 (6.4-10.0)	17. 9.0 (5.8-12.2)	17. 175.5 (154.2-196.9)	Kaur & Dhanju, 2013; Kler et al., 2014
13	8. 19.9 (6.7-33.0)	10. 21.9 (38.1-5.8)	12. 284.0 (412.4-155.7)	Kaur & Dhanju, 2013; Kler et al., 2014
14	-	3. 39.9	11. 305.1	Otero-Pérez, 1998
15	18. 6.6 (0.5-13.0)	13. 14.2 (3.7-51.5)	21. 132.2 (58.4-317.5)	
16	12. 10.4	4. 34.1	22. 121.8	Fu et al., 2013
17	-	-	-	Stoneburner, 1980
18	-	26. 4.5	-	Yin et al., 2008
19	-	34. 1.25	-	Yin et al., 2008
20	1. 478.2	-	-	Bravo et al., 2005
21	4. 24.7	5. 33.8	13. 229.8	Kaur & Dhanju, 2013
22	23. 1.9 (1.1-2.7)	27. 4.4 (0.4-8.3)	-	Lebedeva 1997
23	17. 6.8 (2.6-11.0)	6. 32.8 (3.9-61.7)	14. 205.6 (52.6-358.6)	Kaur & Dhanju 2013; Kler et al., 2014
24	3. 31.2 (8.5-53.8)	2. 66.9 (11.7-122.1)	4. 455.5 (189.2-721.8)	Kaur & Dhanju, 2013; Kler et al., 2014
25	-	30. 2.5	7. 370.7	Morrissey et al., 2005
26	6. 24.2 (5-5)	23. 5.0 (1.5-9.1)	9. 314.6 (112-403.5)	Eeva et al., 2005; Berglund & Nyholm, 2001
27	-	-	-	Berglund et al., 2011; Rainio et al., 2013
28	5. 24.6 (7.9-36.2)	9. 24.4 (3.1-124.8)	10. 314.1 (311-317.4)	Eeva et al., 2009; Rainio et al., 2013; Dauwe et al., 2000
29	-	-	-	Dauwe et al., 2000; Janssens et al., 2003; Dauwe et al., 2004; Eeva et al., 2005
30	7. 24.1 (1.2-60)	16. 12.2 (0.9-112.2)	8. 316.5 (51.1-526)	Berglund et al., 2011; Eeva et al., 2009; Berglund & Eeva, 2015; Rainio et al., 2013; Costa et al., 2012
31	-	-	-	Eeva & Lehtikoinen, 1996; Koivula et al., 2011; Eeva & Lehtikoinen, 2004
32	9. 19.2	8. 25.9	3. 465.0	Kaur & Dhanju, 2013
33	19. 6.4	25. 4.8	1. 704	Turzańska-Pietras et al., 2018
34	-	37. 0.6	-	Cellis et al., 2012
35	-	15. 12.8	-	Espejo et al., 2016
36	-	41. 0.3	-	Ancora et al., 2002; Yin et al., 2008
37	-	38. 0.5	-	Yin et al., 2008
38	-	33. 1.5	-	Yin et al., 2008
39	-	39. 0.4	18. 145	Metcheva et al., 2011
40	-	22. 5.3 (0.4-10.1)	-	Martinez-Haro et al., 2011
41	-	29. 2.5 (0.4-6.0)	-	Martinez-Haro et al., 2011; Martinez-Haro et al., 2013
42	-	33. 1.5	27. 16	Ek et al., 2004
43	20. 6.2* (3.0-9.6)	11. 20.3*(9.3-29.2)	16. 176.0*(48.8-289.8)	Gaba & Vashishat, 2018
44	-	36. 0.7	26. 57.0	Reiter-Marolf et al., 2016
45	-	28. 2.9	-	Yin et al., 2008
46	-	35. 0.9 (0.3-1.6)	6. 419.4	Liu et al., 2006; Yin et al., 2008
47	22. 5.5	7. 28.9	5. 446.3	Fu et al., 2013
48	10. 15.2	-	15. 202.6	Bravo et al., 2005

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Discussion

Spatial variation in heavy metals in gull faeces

Birds have often been used as bioindicators of environmental contamination (Yin et al., 2008; Berglund & Eeva, 2015), but few studies have examined a complete range of heavy metals in gull faeces (see Table 2) and none of them have considered LBBG. In this paper we show that LBBG can excrete high levels of heavy metals in their faeces and that this reflects spatial differences in metal exposure in the environment. As expected, and similar to other studies (e.g., Yin et al., 2008), essential elements (such as Zn) showed low variability among sites. Zinc is an essential trace metal fundamental for physiological functions in all living organisms (Kabata-Pendias, 1993) and is therefore actively regulated and eliminated, at least up to certain concentrations (Muysen & Janssen, 2002). However, other essential elements (such as Cu and Mn), whose physiological functions in vertebrates are also fundamental (Kabata-Pendias, 1993), showed large variation among sites.

In general, patterns of element concentrations were characteristic of each area and were consistent with the main source of pollution in that area. The highest concentrations of As were recorded in faeces from Chipiona Port (where it exceeded the LEL value) and Cetina salt pans, both in the Gulf of Cádiz. These sites sit hydrologically downstream of some of the most heavily mined areas in Europe – i.e., the Iberian pyrite belt (rich in As, Pb, Cu, Zn, etc.) – which has been mined for millennia, depositing large amounts of heavy metal-laden sediments and contaminated water via the Tinto and Odiel rivers, into the Gulf of Cadiz. The Huelva estuary, formed by the union of the mouths of the rivers Tinto and Odiel, in the north west part of the Gulf of Cadiz, is also a heavily industrialized area discharging high concentration of heavy metals into the Atlantic Ocean (Pérez-López et al., 2011). Arsenic load in particular, has been estimated at about 60 kg yr⁻¹ and 2.7 t yr⁻¹ for the Tinto and Odiel rivers, respectively (Sarmiento et al., 2009). This huge amount of highly toxic inorganic arsenic (and other heavy metals) produces a plume of contaminants into the Gulf of Cadiz (Palanques et al., 1995), even reaching the Mediterranean Sea through the Strait of Gibraltar

(Elbaz-Poulichet et al., 2001; Perriñez, 2009; Pérez-López et al., 2011).

LBBG diet at Chipiona Port is based around a natural diet of marine discards (e.g., of gadiforms, clupeiforms, benthonic fish, crustaceans, bivalve molluscs, etc; Oro, 1996), but these can contain high levels of As (Suñer et al., 1999) and other heavy metals (Sarasquete et al., 1997). While the As accumulated in marine food chains is mainly present in organic forms, which are of low toxicity to gulls (Ahrar et al., 2014), these can be degraded back to inorganic As (during the digestive process in a bird), thus increasing toxicity and retention.

The high concentrations of heavy metals in samples from the Cetina salt pans are also likely related to wastewater discharges from surrounding urban settlements (especially for Pb and Cd), and effluents from naval and aeronautical industries (particularly for Zn and Cu, which both surpassed the LEL values) in adjacent areas (Ponce et al., 2000). This salt pan was also part of a large area of marshland which was historically used for hunting (Ruiz & Hortas, 2014), so high levels of Pb in samples from Cetina may be connected with the presence of Pb shot contamination in sediments in the area.

Gull faeces collected at landfills near the Cetina salt pans, (i.e., at VerinSur and Miramundo), contained high levels of Cd and Cr which may be linked to the presence of electronic waste (Adelekan, et al., 2011). Electronic waste is an important emerging problem worldwide (Needhidasan et al., 2014). It contributes approximately 70% of the heavy metals detected in landfill leachates (Li et al., 2009) and gulls may play an important role in transporting this to less contaminated areas (**Chapter 1**). However, our PCA showed an association between Cd-Cu-Co-Pb-Zn-Mo-As and a markedly different heavy metal pattern in the Cetina salt pan with respect to other study locations (including Miramundo and VerinSur), which may indicate that gulls using this salt pan are not feeding in landfills, but instead along the coast of Cadiz Bay.

The expansion of open landfills in Andalusia since the 1980s has driven a major increase in the numbers of LBBG wintering inland in this region. **Chapter 1** identified the 12 most important landfills used by LBBG's, which then roosted in

different wetlands around Andalusia (facilitating metal transport through their guano). These wetlands included many reservoirs and natural lakes, as well as ricefields (in late winter, after the harvest is complete). Fuente de Piedra lake, the most natural site in our study area, is one of the most important wintering roosting sites for LBBG in Spain. These birds feed in surrounding landfills (10.6% of the visits to landfills registered were to the Córdoba landfill 78.3 km away; **Chapter 4**) where they are exposed to high levels of heavy metals (Cd, Cu or Ni; Fig. 3) that could subsequently be deposited in the lake (see section below).

The Doñana ricefields area is located in one of the most important wintering areas for waterbirds in the western palearctic (Rendón et al., 2008), and acts as both a foraging and roosting site for LBBG (**Chapter 1**), with more than 15,000 LBBG here each day during the harvest time (Rendón et al., 2008). Faeces collected in the Doñana ricefields showed the highest values for Mn and the lowest values for Zn. This pattern is consistent with the anaerobic processes occurring in the reduced flooded ricefield soils – which causes increased manganese availability (because of reductions of Mn⁴⁺ to Mn²⁺) and decreased availability of Zn (Fageria et al., 2011). As expected, levels of As in gull faeces were also relatively high in this area (Carey et al., 2019). LBBG in Doñana ricefields feed mainly on alien crayfish *Procambarus clarkii* (Lovas-Kiss et al., 2019), which have been shown to be a good bioindicator of environmental contamination in the ricefields because it bioaccumulates huge quantities of heavy metals, with potential to transfer these to higher trophic levels (Alcorlo et al., 2006; Suárez-Serrano et al., 2010). Contrary to As, levels of Cd were low in the ricefield samples, which is consistent with the reduction in solubility of Cd (i.e., decreased bioavailability) in reduced soil conditions (Moreno-Jiménez et al., 2014).

Overall, our results suggest that LBBG faeces can act as a bioindicator of heavy metal pollution in a variety of habitats (see Gagnaniello et al., 2001, for criteria): 1) we have shown that individuals are able to excrete metals at levels allowing direct analysis without the necessity for pre-concentration; 2) levels of metals in faeces are sensitive to variations in the environment; 3) the LBBG uses a diversity of ecosystems from freshwater to hypersaline, and from natural to artificial; 4) it is a widespread and abundant species; 5) its size, biotope and

behaviour make faecal sampling easy; 6) it utilizes anthropogenic habitats with high concentrations of toxic substances; and 7) census data and movement data can be readily collected, allowing detailed estimates of biovectoring of contaminants into roost sites.

Heavy metal input at Fuente de Piedra Lake

Biological transport of contaminants has typically been overlooked, but increasing evidence suggests that it can be substantial, and even the main pathway for contaminants in many contexts (**Chapter 4**). Gregarious animals that are exposed to contaminants and then move to congregate in specific areas are good candidates to be effective biovectors of contaminants (Blais et al., 2007). LBBG provide an example of a bird with such characteristics: i.e., it is commonly exposed to high levels of contaminants acquired in feeding areas, and then moves to concentrate in high numbers in roosting areas (depositing contaminated excreta at that site).

Here, we provide a detailed estimation of heavy metal inputs by LBBG in one the most important roosting sites in SW Spain, Fuente de Piedra lake (1,350 ha; see Fig. 2 for site details). Movement data has shown that LBBG feed in landfills (e.g., Córdoba landfill here, amongst others) where they are exposed to high levels of heavy metals and then move to Fuente de Piedra lake for roosting (**Chapter 4**). In our approach, we based our calculations on monthly gull counts from seven winters (2010 to 2017) corrected with movement data in **Chapter 4** to estimate time spent at the lake. We found considerable inputs of heavy metals in Fuente de Piedra lake by LBBG, although levels fell below the thresholds for ecological contamination in our samples (Table 1). Loads of Mn and Zn to roosting areas surpassed 700 g ha^{-1} during the last studied winter (in 2017); while Cu reached 250 g ha^{-1} and for the most toxic elements, i.e., Pb, values were 76 g ha^{-1} .

Interestingly, heavy metal loads have increased in recent years, which can be explained by the increase in both the proportion of time spent at the lake and the number of gulls wintering at the lake (**Chapter 4**). LBBG have been gathering in increasing numbers to roost here during the winter since the 1980s, so long

term deposition may be especially important for certain heavy metals (such as As, Pb and Cd), as even low concentrations can be highly toxic (ATSDR, 2019) – in fact, there are no known ‘safe’ levels for metals such as As and Pb. Our results show that, in those parts of the lake where gulls roost, metal accumulation may represent a long term threat to the lake’s functioning. We also did not consider other potential sources of heavy metal load via LBBG, such as pellets, which are also an important source of nutrients in Fuente de Piedra Lake (**Chapter 4**), or moulted feathers, which are known to bioaccumulate large amounts of certain metals (Abbasi et al., 2015). The impacts of excess nutrients in the lake (as previously demonstrated) may be further exacerbated if synergic effects occur with heavy metal loads within this ecosystem (Conde-Álvarez et al., 2012).

Review of heavy metals in bird faeces

Previous studies support the use of faecal analyses as a non-invasive method for environmental biomonitoring (Sánchez-Virosta et al., 2015). However, compared with studies measuring metals in other biotic matrices (e.g., blood, feathers, eggs), our review indicates that there are relatively few studies reporting heavy metals in bird faeces. Nevertheless, among non-invasive methods, metal levels are often higher in excreta than in feathers and eggs (e.g., Metcheva et al., 2011), and easier to sample.

Our review summarized 35 studies, encompassing 45 different bird species from a relatively large geographic area (14 countries from four continents). Most studies were carried out in India (22%), followed by China (19 %) and Spain (16%); the remaining countries were mostly from other parts of Europe. Both essential and non-essential trace elements were reported in bird faeces. However, most studies predominantly focused on toxic metals (i.e., Pb and Cd) and lacked information on other less toxic but important elements (e.g., Co and Mo). Overall, passerines were the most represented group in the different studies (15 studies, 43% of the total). These birds accumulated comparatively high concentrations of metals, probably because of their sedentary nature and urban habits. 57% of species were aquatic birds, which reveals the importance of this group of birds as bioindicators and vectors of heavy metals in aquatic

environments. Among aquatic birds, penguins were the most studied. Gulls were only studied in four previous cases (our study being the fifth in gulls, and the first for LBBG and inland wetlands).

We found important differences in concentrations of heavy metals between bird species. The highest levels of Pb, Cd and Cr were found in red-wattled lapwing *Vanellus indicus* and were related with more urban environments and anthropogenic diets (Kaur & Dhanju, 2013). Faecal concentrations of heavy metals was also highly site dependent, with studies from India showing the highest values. However, it is difficult to draw general patterns, because intrinsic factors also affect levels of heavy metals including body size, age, metabolism, physical condition, different feeding and accumulation strategies (Walsh, 1990).

In general, levels recorded in our study are not that high in comparison with other anthropogenic scenarios, but they are high in comparison with other gull studies. The levels of As here fell within the top 10 avian studies with the highest values. This is important because of the high toxicity of this metalloid, ranking No.1 on the priority substances list (ATSDR, 2019). Regarding Pb, (ranked 2nd as a priority toxicant; ATSDR, 2019), the levels from our study fall below most of our reviewed studies, and are lower than in other gull species that share similar dietary requirements (Peterson et al., 2017).

The scarcity of studies reporting heavy metals in faeces makes it difficult to understand under which conditions heavy metal levels may vary. As we stated before, heavy metal levels are site, species and trophic level dependent, which can result in significant variations, making comparisons and interpretation difficult (Bosch et al., 2016). Broad spatial scale studies, both between and within species, using standardised protocols and sampling strategies, would be very useful to allow cross-study and species comparisons to provide better relationships between environmental pollution and metal levels in avian faeces.

Future work

Given the increasing presence of contaminants in the environment, it is more critical than ever to identify potential bioindicator tools for toxic elements in different environments, and to examine biologically-mediated pathways. In this study, we provide the first data supporting the role of LBBG as bioindicators and biovectors of heavy metals in a variety of aquatic ecosystems via their faeces. **Chapter 1** used movement and count data to identify 10 functional units (modules) within a connectivity network for LBBG in Andalusia, one of which was centred on Fuente de Piedra. Most of these units are centred around reservoirs, lakes or other wetlands used by large numbers of LBBGs that feed at landfills. Faecal analysis would allow estimation of contaminant inputs into key wetlands in any of these other functional units. Likewise, a similar approach could readily be adopted for other gull species with similar movement data (e.g., Ahlstrom et al. 2019; Navarro et al., 2016).

It is also a priority to understand species responses to environmental change to predict biotransport and pathways of contaminants. Moreover, with the numerous anthropogenic pressures facing the environment, and the increasing availability of anthropogenic food resources for gulls, contamination burden transported by birds to natural areas is expected to increase. New methodological approaches can improve our ability to study bioaccumulation and contaminant transport by biovectors. Incorporating movement data of frequently tracked birds, such as LBBGs, will allow us to increase our ability to quantify biologically mediated contaminant flux and its implications for ecosystems and human health. From an ecosystem and health perspective, more attention should be paid to the role of gulls as vectors of pollutants in the long term, particularly in inland wetland ecosystems (used by millions of gulls across North America, Winton & River 2017), which are much less studied when compared with marine and other terrestrial ecosystems.

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Supplementary materials

Table S1. Limit of Detection (LOD) and Limit of Quantification (LOQ) for each of the ten elements. Certified (CERT), average (AVE) and recovery (REC) levels for both Lobster Hepatopancreas (LOB) and Bushes, Branches, Leaves (BBL) for each of the ten elements.

	As	Cd	Co	Cr	Cu	Mn	Mo	Ni	Pb	Zn
LOD	140.7	7	24.2	54.7	33.3	18.7	66.2	37.1	462.5	253.4
LOQ	469	23.2	80.8	182.2	110.9	62.3	220.6	123.8	1541.5	844.6
CERT LOB	21600 ± 1800	26700 ± 600	510 ± 90	770 ± 150	106000 ± 10000	13600 ± 1200	950 ± 100	2500 ± 190	<LOD	180000 ± 6000
CERT BBL	950 ± 120	140 ± 60	390 ± 50	2300 ± 300	5200 ± 1200	58000 ± 6000	260 ± 40	1700 ± 400	7100 ± 1100	20600 ± 2200
AVE LOB	24736.9	27286.75	497.5	773.4	95863.4	12402.95	960.35	21.92.35	NA	186497.25
AVE BBL	1180.85	235.8	327.4	1464.85	4444.6	55457.7	214.95	1548.3	7231.5	22397.7
REC LOB	114.5	102.2	97.55	100.4	90.4	91.2	101.1	87.7	NA	103.6
REC BBL	124.3	168.4	83.95	63.7	85.45	95.6	82.7	91.1	101.85	108.75

Table S2. Anova results (F, degrees of freedom and p-value) among locations for the ten elements (As, Cd, Co, Cr, Cu, Mn, Mo, Ni, Pb and Zn) studied.

Element	F	df	p
As	19.3	6	< 0.0001
Cd	14.74	6	< 0.0001
Co	20.18	6	< 0.0001
Cr	21.35	6	< 0.0001
Cu	8.48	6	< 0.0001
Mn	52.05	6	< 0.0001
Mo	52.49	6	< 0.0001
Ni	33.65	6	< 0.0001
Pb	9.05	6	0.01
Zn	5.33	6	< 0.0001

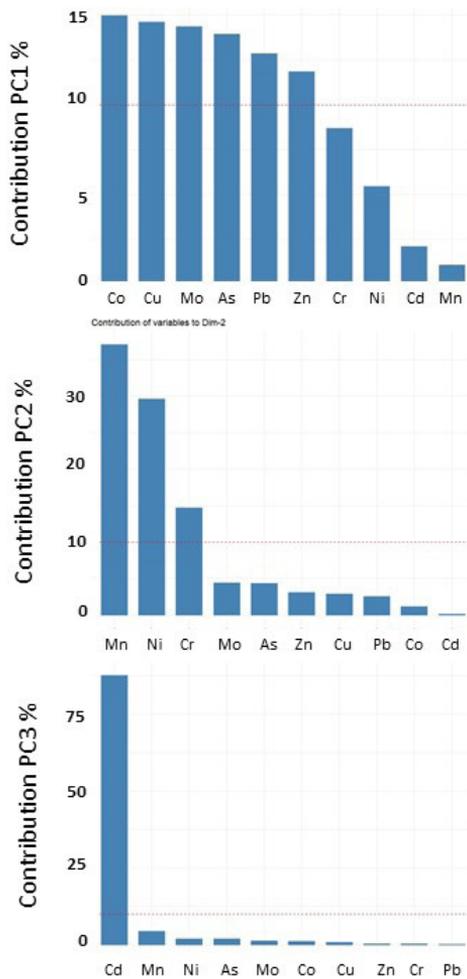


Fig. S1. Contribution of each element to the two main axes of the principal component analyses (PCA).

General discussion

Synthesis

This thesis expands our understanding of the importance of waterbirds in biovectoring of contaminants and other dispersal processes, especially those related to exploiting anthropogenic environments and their close connections with aquatic systems. How waterbirds can connect different environments has not been paid much attention in the past, and previous studies were largely limited to the lack of detailed data on waterbird GPS movements within the environment.

This thesis is based on how combining GPS gull tracking with network analyses can identify and quantify connectivity links, to determine the main routes for biovectoring and dispersal (**Chapter 1**). Depending on what precisely is being transported (e.g. seeds, nutrients, heavy metals), the source and the strength of the connection were crucial to quantify the importance of the vector in the process. In this chapter, I could show how waterbirds are involved in biovectoring and dispersal among anthropogenic (e.g. landfills, ricefields, ports) and natural habitats within Andalusia. The generalist feeding behaviour of the lesser black-backed gull (LBBG) allows them to take advantage of human resources, but also brings consequences of “disservices” that have impacts in aquatic ecosystems and other agricultural landscapes when depositing guano (weed dispersal, eutrophication, external pollution). Although we studied the whole Andalusian region, this study helped to scale down the transport events, as the probability of movements between environments was limited to 60 km within a certain functional unit in which the gulls combine different behaviours (mainly foraging and roosting). The application of such connections with field studies allowed me to determine the origin and quantification of such “disservices”, and to propose measures to maintain aquatic ecosystems in a healthy state.

Seed dispersal services have been widely reported in frugivorous birds in a variety of environments. The dominance of such studies reflect the widespread assumption that only plants with fleshy fruits are dispersed by birds. However,

General discussion

a more recent trend in research has demonstrated that waterbirds are able to disperse a wider range of plant species (Lovas-kiss et al., 2018a; Reynolds et al., 2015; Soons et al., 2016). These plants do not have an endozoochory syndrome, but instead have been assigned to other syndromes related to other means of dispersal (e.g. wind, water). Among seed dispersal studies, agricultural (and artificial) landscapes have been less investigated than natural environments, and how their plant communities are dispersed by birds is less understood. In **Chapter 2**, I showed the potential for seed dispersal within the ricefields of Doñana by using two model species with different body sizes and bill morphology (the LBBG and the white stork), but similar feeding behaviour. At least in this artificial environment, the community of plants dispersed by these vector species with different body sizes was equally broad and overlapped greatly. The great majority of plants dispersed were agricultural weeds, and some presented herbicide resistant populations, which may have implications for dispersal towards other agricultural landscapes. This redundancy in dispersal vectors suggests that other avian vectors that feed in a similar manner within the ricefields are likely to make a similar contribution to seed and weed dispersal.

Furthermore, I applied the GPS gull data to develop seed dispersal models to predict seed shadows for weed deposition within the study area more realistically (**Chapter 3**). In this way, I quantified the medium to long distance weed dispersal events carried out by gulls within and beyond the ricefields. In this case, the length of time the vector was able to retain a seed within its digestive tract determined the weed dispersal distances whereas the movements of the vector itself were involved in the spatial deposition patterns. Gull daily movements determined the directionality of the weed dispersal, but also exceeded the weed dispersal distances in comparison to other dispersal means (e.g., wind, water). Dispersal distances were modelled between hundred meters and hundred kilometres, with many seeds deposited in suitable environments (including lakes as far away as Fuente de Piedra), so weeds could establish and grow. This model could be readily adapted to predict seed dispersal in other study areas when GPS information data is available for a vector species.

Previous studies have quantified the amount of external nutrients (N and P mainly) deposited in roosting sites by waterbirds (guantrophication). If the frequency of guano deposition is high (because of a repetitive movement behaviour and/or the high number of individuals), eutrophication may interfere with the balance of the aquatic ecosystem. Here, I studied the example of Fuente de Piedra, an important natural lake where the LBBG roosts daily during winter (**Chapter 4**). Through GPS data, I adapted previous nutrient loading models to correct the time that gulls spent at the lake, but also to correct how censuses were made when part of the gull population had already departed towards their foraging sites. Furthermore, I could identify four main landfills (and their relative nutrient contributions) where gulls were foraging daily before roosting at the lake.

This behaviour may bring other biovectoring implications to the lake, such as heavy metal deposition (**Chapter 5**). In **Chapter 5** I could also determine that the heavy metal presence in gull faeces strongly varied among sites and depended on different pollution sources related to historic activities (e.g. mining and industries at Cetina salt pans, fisheries at Chipiona port or human activities such as waste deposition in landfills). Those sites are “sources” for heavy metal ingestion when gulls forage in ports and landfills, that will be potentially deposited in aquatic “sinks” due to biovectoring processes. Both **Chapters 4** and **5** are clear examples of how the behaviour of the gulls beyond the boundaries of an ecosystem can affect an aquatic system like Fuente de Piedra, which is a closed basin lake where water output is only via evaporation. This means that external pollutants, such as nutrients and heavy metals, are potentially deposited and accumulated within the lake during successive years.

Thesis implications

Stablishing connectivity networks of waterbirds moving between habitats helps us to visualize where, when and in which direction the biovectoring and other dispersal processes mediated by waterbirds may occur. For example, the dispersal of plants lacking fleshy fruit, has been paid less attention than

other frugivorous dispersal, but waterbirds have been proved that are effective dispersers of a wide range of plant species that are not assigned to endozoochory syndromes in different environments.

In **Chapter 1**, Doñana ricefields were the most central node that contributed the most to maintain the overall connectivity generated by gulls along Andalusia. While foraging at the ricefields, gulls and white storks are able to ingest and effectively disperse a wide variety of seeds that are lacking fleshy fruit within a ricefield area (**Chapter 2**). Many generalist plant species (e.g. *Spergularia marina*) and other wetland associated plants (*Lemna gibba*, *Lemna minor* and *Chara* sp.) were reported to be effectively dispersed by gulls and storks. Many other waterbirds (e.g., *Egretta garzetta*, *Bubulcus ibis*) share resources and feeding behaviour within the ricefields during harvesting season and would provide the same dispersal services to many plants and other aquatic organisms. Therefore, the seed dispersal service provided by waterbirds within and beyond the ricefields seem like a frequent event which may enhance the connectivity of many plant species able to survive waterbird ingestion.

However, exploiting anthropogenic resources can bring negative consequences (e.g. the dispersal of alien species or weeds associated to crops). In such cases, the functional effects of waterbirds to ecosystems can be considered “disservices”. In **Chapter 2**, almost 40% of the seeds encountered through faecal analyses in gulls and storks were weeds associated with rice crops and 20% were alien species. The majority of the weeds dispersed by gulls remained within the ricefield area (**Chapter 3**), so gulls may contribute to the genetic homogenization of weed plants, as has been shown for the snail *Physa acuta* (van Leeuwen et al., 2013). However, weeds normally present a broad range of habitat requirements and are able to establish and grow in many habitats beyond ricefields. Other irrigated agricultural areas, as well as natural and artificial wetlands in the surroundings, were identified as suitable deposition for weeds (**Chapter 3**).

Beyond weeds, waterbirds can potentially disperse many other propagules associated to agricultural landscapes. In ricefields, a range of alien species such as the waterwort *Bergia capensis* (and a wide range of exotic macroinvertebrates

such as snails, ostracods and bryozoans) were found to be dispersed by gulls and storks (**Chapter 2**). In fact, I found an adult individual of the alien gastropod *Physa acuta* to be successfully dispersed by gulls through pellet deposition (results not shown).

Additional data collected during my thesis show that a wide range of macroinvertebrates can be effectively dispersed by gulls (results not shown, see also Lovas-Kiss et al. 2018a). Ephippia (resistant eggs from Cladoceran) from *Machrotrix rosea* were found to survive gull digestion and hatch. Other cladoceran species did not hatch but were found intact after gull digestion, so gulls are potential vectors for their dispersal. The fact that gulls, and waterbirds in general, can connect different aquatic ecosystems through their movements (**Chapter 1 and 3**), suggests they provide dispersal services to a wide variety of aquatic species, helping them to colonize new environments and to escape from the unfavourable conditions. For example, I was able to show that gulls may be connecting successfully Doñana ricefields with several reservoirs, but that they can also reach as far as Fuente de Piedra, an important roost site for gulls that is 118 km distant from the ricefields. Within this context, the “positive service” of gulls as propagule dispersers may compensate for the “disservices” generated by exploiting artificial landscapes and transporting weeds. In a global change context in which wetland degradation is increasing, the ability to escape to new suitable environments can be crucial from a population point of view (Reynolds et al., 2015).

Waterbirds also exploit resources from other anthropogenic habitats, such as landfills which can be a source of nutrients and heavy metals, but also for seed dispersal of commercial plants present in the waste such as olives or tomatoes (Calvino-Cancela, 2011). Biovectoring processes may have effects at several spatial scales and target in a wide range of ecosystems. In case of gulls, the effective range of biovectoring occurs normally within a 60 km radius and within a functional unit. A functional unit includes at least one aquatic system that acts as roosting site and one anthropogenic site (e.g. landfills, ports) that acts as foraging site (**Chapter 1**). Therefore, the main sources for contaminant transport by gulls can be determined in each case. For example, the functional unit around Fuente

de Piedra lake involves several rubbish dumps up to 80 km away (**Chapter 4**). The combination of GPS data, network analysis and faecal analyses to determine nutrient and metal content provides a realistic quantification of the impact that gulls are generating at a specific roosting site (**Chapters 4 and 5**).

The same procedures may be applied to other functional units with similar characteristics. For example, the main rubbish dump in Linares (Jaen province) is highly connected with three reservoirs up to 22 km away (Giribalde, Guadalén and Fernandina). Similar amounts of nutrients and heavy metals, as well as other contaminants, may be expected to be deposited in these reservoirs as in Fuente de Piedra. However, the nature of the aquatic system is important to be taken into account, as reservoirs have a water renewal rate higher than in Fuente de Piedra (closed basin lake). Furthermore, the roosting behaviour by the gulls is another determinant. In Fuente de Piedra, gulls are known to roost in islands that emerge in the middle of the lake when water levels are intermediate (**Chapter 4**). Therefore, the concentration of metals, for example, will be higher at such roosting places than in the rest of the Lake (we can see metal estimations for roosting sites in **Chapter 5**).

Management perspectives

Weed management

The Andalusian region has a high percentage of its surface covered by intensive agriculture. According to Corine Land Cover 2012, 60% of the land use within the region is dedicated to agriculture, with dominance of olive trees and irrigated agricultural lands other than ricefields. Homogeneity in agricultural landscapes provides plenty of suitable habitats for weeds to be deposited and established because of their generalist nature and their adaptability to grow in a wide variety of crops (Bourgeois et al., 2019). A more heterogeneous landscape configuration, in which agricultural fields are combined with wetlands and more natural environments, will increase the chances of other species to be deposited by gulls in suitable habitats, and thus, the services provided by gulls will compensate their disservices.

Weed expansion has implications for the dispersal of new genotypes that are resistant to herbicides (Farmer et al., 2017). At high densities, weeds compete with rice production and are considered one of the main causes of yield loss in rice production (Ferrero & Vidotto, 2007). Without any weed control, yield loss can increase up to 90%. To avoid yield loss, weeds are managed through the use of herbicides (Tian et al., 2020). The use of chemical herbicides accounts for around 80% of all used pesticides in crops, and an estimated €110 million are spent each year in Europe (Ferrero & Vidotto, 2007). From an agricultural perspective, knowledge on weed dispersal will help to prevent and manage weeds within crops, while avoiding further economic losses.

Rubbish dumps and waste generation

As has been seen throughout this thesis, gulls rely extensively on waste resources from rubbish dumps (or landfills) for daily foraging within the Andalusian region (**Chapters 1 and 4-5**). Many birds have changed their migratory patterns because of the availability of new resources, since birds no longer have to migrate further to find resources. There are examples of storks that do not migrate to Africa anymore (Arizaga et al., 2018), or even LBBG individuals which used to travel to Africa which now remain in Andalusia (Shamoun-Baranes et al., 2017; Alerstam, 1990). In the 1980s, open landfills or rubbish dumps were established in Spain to avoid rubbish burning (Tortosa et al., 2002). For example, the landfills where gulls forage when they roost at Fuente de Piedra lake opened progressively from 1986 (Córdoba landfill) to 2006 (Matagrande landfill), which has promoted the increase of LBBG population at Fuente de Piedra in the last decades.

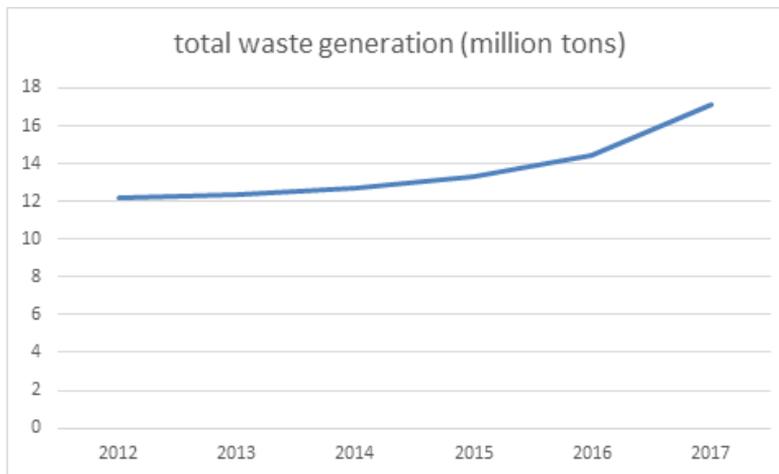


Figure 1. Total waste production increasing trend in Andalusia region from 2012 to 2017. Source: Plan Integrado de Residuos de Andalucía 2019.

According to data from the Integral Plan of Waste (PIRec, 2019) from Andalusia, 17.1 million tons of waste were estimated to be generated in 2017 (Fig. 1). More specifically, this was 19% more than the previous year 2016. From such waste, urban waste is the second category that accounts most for waste generation (28% of the total waste within Andalusia). In 2016, 4.2 million tons of urban waste were estimated to be generated. From the total of urban waste generated, only 10% was collected and separated selectively according to their category of plastic, compost, glass, oil, cardboard, wood and others (Fig. 2). The remaining 90% corresponded to “general waste” destined for the biological-mechanical waste treatment plants (so-called in this thesis rubbish dumps or landfills) that recycle and separate some of the materials (metals, plastics, organic sources) from others that cannot be separated. Those resources available in rubbish dumps are the ones available for many birds to feed on, as the waste is accumulated openly before processing (Duhem et al., 2008).

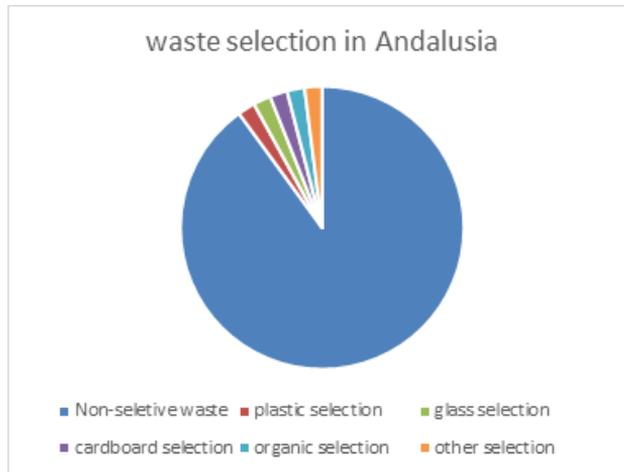


Figure 2. Percentages of waste selection in Andalusia. Grey correspond to non-selective waste. Yellow to plastic packaging, green to glass, blue to cardboard, red to organic waste, and light blue to other types. Source: Plan Integrado de Residuos de Andalucía 2019.

Future perspectives in research

The contaminants associated with landfills are highly variable. In this thesis, I only focused on the biovectoring of nutrients, heavy metals and metalloids as examples of external loading. However, the effect of such excess of nutrients and heavy metal deposition on ecosystem dynamics and balance is not clear. Further experiments or models showing the effects of pollution in the water column would be necessary in Fuente de Piedra. Same examples of guantrophication models could be applied to other aquatic systems with high number of gulls (e.g. reservoirs). The use of other sample types (e.g. blood, feathers) as bioindicators of contaminant exposure should be further investigated. At the same time, studies related to the quantification of heavy metal exposure in soils and water will help to determine the degree of certainty as bioindicator of each sample and bird species.

Other sources of pollution should be considered in order to understand completely the biovectoring role from gulls to aquatic systems. Previous evidence

General discussion

showed that plastic biovectoring was very common in many aquatic systems in Andalusia. For example, in 23 gull pellets sampled in Fuente de Piedra lake, 11 (48%) presented plastics (results not shown). Therefore, gulls are likely contributing to plastic deposition in wetlands. Furthermore, landfills are sources of other less evident contaminants, such as antibiotic resistant bacteria (ARB) and Persistent Organic Pollutants (POPs). Only specific analyses to determine and quantify such pollutants in gull excreta will throw further light on the biovectoring loads by gulls.

The scale of connectivity has important ecological implications for dispersal and biovectoring. At a local scale, other connections within the connectivity network were not taken into account, and could be important in biovectoring at shorter distances. For example, Cadiz province was isolated from the network in **Chapter 1** but there were not enough GPS movements to reflect entirely the connectivity between habitats. According to census data and **Chapter 5** results related to heavy metals at different sites in Cadiz province (Cetina saltpan, Chipiona port, two landfills), the gull activity in this area seems to be high. At large scale, there are likely to be many other waterbirds involved in the seed dispersal processes within and from ricefields (white storks, herons and egrets, ducks, shorebirds, etc), and many of such birds are migratory. Therefore, the broader scale for long distance seed dispersal should be taken into account. GPS data associated to other waterbirds (e.g. white stork) with similar behaviour as gulls could be applied to generate network analyses and dispersal models similar to the ones carried out in this thesis.

Weed dispersal within and beyond the ricefields is a common event that can be repeated daily during harvesting season and improves the dispersal distances greatly in comparison with other means of dispersal. However, there is no information related to dispersal events after harvesting (after December). According to connectivity and GPS data, many gulls remain within the ricefields but their diet or their dispersal potential is unknown. Sampling after December within the ricefields will help to clarify that. Gulls also feed in other agricultural landscapes beyond ricefields (e.g. olives, cereals), so similar models could be applied. Seed dispersal models and GPS data can be combined with connectivity

analysis to determine the role of individual variation in seed dispersal. For example, some individual gulls are specialized to remain within a narrow range with low mobility, whereas other more generalist individuals exploit broader range of resources and are more mobile, likely contributing more to seed dispersal distances.

Furthermore, the contribution of waterbirds to genetic exchange by seed dispersal are not clear either. Developing models that account for gene flow (especially of herbicide resistant genes) will help to understand the role of waterbirds in weed dispersal and their implications for agricultural management. Dispersal mechanistic models could be applied to predict an invasion from an alien species. If different waterbird are able to disperse same invasive species, for example, the ability to spread will increase at different scales.

Conclusions

Chapter 1

- LBBG provide important connections between terrestrial and aquatic habitats due to their high mobility and generalist behaviour. Doñana ricefields and various rubbish dumps performed as central nodes to maintain connectivity in the whole Andalusian region, although such connections changed seasonally together with changes in habitat use.
- High connectivity implies transport of organisms, nutrients and contaminants between different habitats by LBBG biovectors and dispersers. Most transport occurs within 60 km distance and within ten functional units, and this has important management implications.

Chapter 2

- Gull and stork excreta analyses showed that avian endozoochory is a major means of dispersal for at least 21 plant taxa within an agricultural ricefield landscape. Eleven of these taxa germinated under laboratory conditions, eight plant species are considered weeds, four of them are alien species, and only two have a fleshy fruit. Toadrush *Juncus bufonius* was the dominant species, accounting for 49% of seeds recovered.
- There is a strong overlap in the communities of non-fleshy fruited plants dispersed by the LBBG and white storks, although they are very different in size. This suggests that waterbird-plant dispersal networks are different from frugivore-plant networks, with greater functional redundancy.
- Endozoochory by migratory waterbirds has major implications for plant distributions in a rapidly changing world, especially for how plants respond to climate change, land use transformation or introductions of alien species.

Chapter 3

- For *J. bufonius* and three other common weeds within the ricefields, LBBG performs an important service in seed dispersal. According to mechanistic models, an important fraction of the seeds dispersed by LBBG will remain within the ricefields, due to their vast area and temporal dynamics. From 7 to 9% of the seeds are estimated to be deposited beyond ricefields, with important implications for population dynamics and weed management.
- Such weeds do not show classical endozoochory dispersal syndromes, but LBBG as a vector provided maximum dispersal distances several orders of magnitude greater than those predicted from their dispersal syndromes. Furthermore, spatial patterns of seed deposition depend greatly on the daily movements based on GPS information.
- Weeds are predicted to be dispersed beyond a hundred kilometres, even reaching wetlands such as Fuente de Piedra lake, an important roosting lake for LBBG in Andalusia. This connection implies that not only weeds may benefit from LBBG, but also many aquatic invertebrates.

Chapter 4

- External nutrient loading to Fuente de Piedra lake is a good example of how inland closed basin lakes can be impacted by birds from outside of the watershed. Changes in land use have driven birds to exploit anthropogenic resources such as landfills, and have triggered an increase in nutrient loads in aquatic ecosystems through guano deposition.
- Four landfills were identified as the main sources of nutrients, where gulls were foraging daily before roosting at Fuente de Piedra lake, according to GPS data. Every landfill had a different contribution to nutrient loading depending on the relative time spent by gulls foraging at the landfill. GPS data were also used to demonstrate that about 69% of the gulls had already departed from Fuente de Piedra lake towards foraging sites before censuses began. Therefore, gull counts

Conclusions

were underestimated, but censuses could be corrected.

- An estimated average of $10.17 \text{ kg N ha}^{-1} \text{ year}^{-1}$ and $2.07 \text{ kg P ha}^{-1} \text{ year}^{-1}$ were imported to this closed-basin lake by gulls each winter, with the highest values recorded in the winter of 2016–2017. Nutrient inputs to the lake depend partly on the proportion of the day that gulls spend there (estimated by GPS data) and the number of gulls present that winter. Gull guano was the most important winter source of P to the lake. Regurgitated pellets have been ignored as a source of nutrients in other guanotrophy studies, but in this study provided more P than faeces.

Chapter 5

- Faecal analysis was used to evaluate the potential of LBBG as a bioindicator of environmental contamination in Andalusia. Several elements (As, Cu, Mo, Pb, Zn) exceed (between 2 and 11 times) the Lowest Effect Level (LEL) values (threshold for soil environmental pollution) in some locations.
- There was strong spatial variation, related to the main pollution sources associated with the different sites. Faeces from Chipiona Port (Gulf of Cádiz) showed the highest levels of As; Cetina salt pans (Gulf of Cádiz) ranked first for Pb, Zn and Mo, which was consistent with historic mining and industrial pollution; Doñana ricefields showed the highest levels of Mn, a highly available element in flooded areas; while landfills ranked first for Cd, Co, Cr, Cu and Ni, potentially associated with electronic waste.
- At Fuente de Piedra, metal inputs by LBBG have increased in recent years based on the same models used for nutrients nutrient loading. Long-term deposition (e.g., of Pb) may impact aquatic communities and ecological processes in the lake.

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I am **Víctor Martín Vélez**, born and raised in Madrid, where I studied Biology at Autonomous University of Madrid. In 2011, I took the great choice of going on Erasmus to Aarhus, Denmark. Both universities changed my professional and personal life, so I decided to start travelling and study a wide range of ecosystems and species. At the point that I can consider now myself as a “generalist”. In Denmark, I started working with GIS and SDMs of iberian macroinvertebrates. My degree project in Madrid was based the habitat selection and distribution modelling of an endemic lizard in South Spain.

After finishing my degree in 2013, I headed to Wageningen, The Netherlands, where I took a master on Forest and Nature Conservation. My master project was based in Kashmir (Indian Transhimalayan) where I studied the altitudinal gradient of macroinvertebrate distribution within high mountain lakes. Afterwards, I headed for my internship to Dominican Republic to study the diet and spatial distribution of endangered iguanas.

I finished my master in 2015 and I took one year-break travelling around Chile, Brasil and Mexico. Afterwards in 2016, I decided to move to Seville to start my current PhD thesis. During those 4 years, I continued my academic training in R, GIS and statistics, and I could go on stay to the Netherlands to collaborate with NIOO and University of Amsterdam. Furthermore, I could assist in teaching at University of Alcalá de Henares. I discovered the world of education and currently I am carrying out a Master in teaching training, so I can combine education with scientific research.

More about me:

<https://scholar.google.es/citations?user=WSpAOjcAAAAJ&hl=es&oi=ao>

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