# Movement and foraging ecology of partially migrant birds in a changing world

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# Contents

**Title page photos***,* clockwise from left: *White stork with a data logger; cork oak savannah and rice field in the Setúbal district, Portugal; lesser kestrel feather stable isotope samples on the mass spectrometer; white storks on Evora landfill, Portugal.*

## Abstract

Globally, migratory behaviour is changing in response to climatic and anthropogenic change. In recent decades, previously wholly migratory species started forming resident populations in the breeding area. Partially migratory species with resident and migratory individuals in the same population provide an opportunity to understand the causes and consequences of changing migratory behaviour. This study focuses on the influence of climate and food availability in determining movement and behaviour patterns of birds in populations that have recently become resident.

The white stork *Ciconia ciconia* recently established a resident population in Iberia, likely facilitated by the availability of abundant anthropogenic food resources including landfill and the invasive red swamp crayfish *Procambarus clarkii*. Movement data from individual white storks fitted with GPS data loggers showed that year-round nest use by resident individuals dictates many aspects of foraging behaviour, including frequency of landfill use and foraging range. Storks visited landfill from nests further away than previously expected (~48 km). High productivity near landfills has likely influenced the rapid population increase observed in recent decades, however breeding success in colonies far from landfill, particularly those located near rice fields, is low. This suggest that the imminent closure of landfills, due to EU directives, will have significant impact on white stork numbers.

Many species are still too small to be tracked with GPS tags, so stable isotopes can be used to identify breeding and wintering quarters of migratory birds. Stable isotopes were used to separate residents from migrants in a partially migratory population of lesser kestrels *Falco naumanni*. Carbon isotopic composition of feather samples indicated that birds completing their moult in Africa could be identified. However, resident birds could not be separated from birds that moulted in Iberia prior to migration. This emphasises the need to understand moult timing and sequence to correctly interpret stable isotope data.

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My PhD has been a thoroughly enjoyable, exciting experience and it is entirely due to you all.

# Chapter 1

## Introduction

**Photo** *Left: White stork nests at high density near landfill, Coimbra, Portugal, Right: white stork chicks hatching.*

### 1.1. Species response to recent climate and environmental change

Globally, species are having to respond to increasingly rapid climate and environmental changes. Global climate has warmed by approximately 0.6$^{0}$C during the 20th century (IPCC 2014), with recent decades (since the late 1970s) likely being the warmest in the last millennium (Jones *et al* 2001). At regional scales climatic change is very heterogeneous and manifests in a variety of ways, including increases and decreases in temperature, and/or precipitation, and changes in the timing, predictability and intensity of climatic events (Walther *et al* 2002). Rapid climate change or climatic extremes can lead to rapid changes in habitats and their ecological communities.

Species have responded to changing conditions in a variety of ways, including distribution shifts (range expansions/contractions) to track preferred climatological and environmental niches (Parmesan and Yohe 2003, Parmesan 2006, Hickling *et al* 2006). This has led to changes in local community structure, biodiversity and species richness, including species invasions (Cheung *et al* 2009, Bellard *et al* 2013) and outbreaks of pathogens (Pounds *et al* 2006). Plants and animals are also changing the timing of seasonal events (phenology), such that leaves and flowers are opening earlier (Peñuelas and Filella 2001) and breeding is occurring earlier in the year (Crick and Sparks 1999, Forchhammer *et al* 1999, Walther *et al* 2002). This has high potential for a mismatch in timing between breeding season food demand and peak availability of food resources, particularly in migratory species (Sanderson *et al* 2006, Visser and Both 2005, Post and Forchhammer 2008, Jones and Cresswell 2010), which can have consequences for fitness, breeding success and overall population dynamics.

Coupled with climatic change, significant environmental changes associated with anthropogenic activities are also a major threat to biodiversity, including agricultural intensification, habitat degradation, loss and fragmentation (Donald *et al* 2006, Sirami *et al* 2008, Wilson and Cresswell 2006). This impacts the dispersal ability of species to track climatic change and is particularly affecting migrants. This is because migratory birds visit multiple countries and potentially have to respond to different rates and extents of change in the breeding, stop over and wintering sites. Consequently, the populations of many migratory species are declining rapidly (Saino *et al* 2011, Vickery *et al* 2014). In a changing world, the flexibility to adapt is key, and there is evidence to suggest that phenotypic plasticity to the timing of reproduction and migration is being positively selected for, and intensifying, as a result of climatic change pressures (Nussey *et al* 2005).

### 1.2. Changes in migratory behaviour

Migratory behaviour is changing in response to climatic and anthropogenic change. Many avian migratory species are changing their migratory phenology by returning earlier and leaving later from the breeding areas (Cotton 2003, Marra *et al* 2005, Gordo and Sanz 2006), they are also changing their winter distribution and migration distances by wintering closer to their breeding grounds, known as short-stopping (La Sorte and Thompson 2007, Visser *et al* 2009, Parkin and Knox 2010, Elmberg *et al* 2014). Birds are also changing migratory behaviour and population distribution in response to anthropogenic food supplementation from sources including landfills, fisheries waste, wildlife tourism and bird feeders (Tortosa *et al* 2002, Orams 2002, Yorio and Caille 2004, Robb *et al* 2008).

In recent decades, even more profound and fascinating changes to migratory behaviour have occurred, with individuals from migratory species abandoning migration completely (Newton 2008). In southern Europe, particularly the Iberian Peninsula, several previously entirely migratory species have become increasingly sedentary, with significant numbers of individuals overwintering in the breeding area with such regularity that they are now considered to have established resident populations (Berthold 2001, Atlas Team 2008, SE0/Birdlife 2012). The Mediterranean has been indicated as one of the world's most responsive regions to climate change, a primary climate change hot-spot, based on A1B, A2 and B1 IPCC future emission scenarios (Giorgi 2006). Recent changes in migratory behaviour are likely associated with climatic change already observed in this region (Perez and Boscolo 2010). In fact, there is evidence to suggest that, in recent decades, climatic overlap between the Iberian Peninsula and former African wintering areas has increased, and that species that have recently formed overwintering populations are selecting wintering locations in Iberia that are warmer and more similar to their former African wintering areas (Correia 2014).

Temperature therefore seems to be the main factor facilitating the establishment of resident populations of formerly migratory species in Iberia. Migration is a solution to the seasonality of local climatic conditions and limited food supply (Newton 2008). Milder winter temperatures reduce winter mortality and increase food abundance by promoting the earlier emergence of insects (Bale *et al* 2002). Additionally, in the Iberian Peninsula, supplemental food from anthropogenic sources, including landfill and invasive escape species, are buffering the impacts of climatic variance and having a major influence on population size, distribution and migratory decisions in some generalist species (Tortosa *et al* 2002, Navarro *et al* 2010, Tablado *et al* 2010, Sanz-Aguilar *et al* 2015).

Despite these recent, considerable and fascinating changes in migration, the ecology of formerly wholly migratory species that have become resident is not well understood. In particular, the behaviour and movement patterns of resident populations during the non-breeding season has not been well studied. Greater understanding of the role of food availability and climate in influencing the distribution, movement behaviour and productivity of recently resident populations will increase our capacity to predict how partially migratory species may respond to future climatic and environmental change and will assist in the design of effective conservation strategies. Additionally, the imminent closure of all open-air landfills (due to EU directives) offers a rarely available opportunity to study the impact of an abrupt change in food supply on distribution, habitat selection, population dynamics and migratory decisions.

## 1.3. Thesis structure

### 1.3.1. Concepts

This study considers movement and behaviour of two partially migrant species that have both recently formed resident populations in the Iberian Peninsula, the white stork *Ciconia ciconia*, and the lesser kestrel *Falco naumanni*. I focus particularly on the white stork because its large size enabled individuals to be equipped with high precision GPS data loggers to allow high resolution analysis of habitat use, movement and behaviour. I was also fortunate in being able to combine a historic data set of white stork productivity from Portugal with my own fieldwork data.

White storks are known to feed intensively year round on landfill (Figure 1.1) and the invasive red swamp crayfish which, coupled with milder winters, has enabled individuals to overwinter in increasing numbers in Iberia. Residency, coupled with changes in drought conditions in the Sahel are thought to be important in reversing the strong population decline occurring until the 1970s (Janss and Sanchez 1977, Kanyamibwa *et al* 1990, Tortosa *et al* 2002). Little is known about the movement

**Figure 1.1** *White storks foraging in high density on Beja landfill, Portugal*

and behaviour of resident storks in the Iberian Peninsula during the winter, particularly in relation to landfill use, so this study represents an excellent opportunity to explore the effects of an abundant, artificial food supply on wintering and breeding ecology. The climatic drivers governing landfill use, are also unknown. These are particularly important to understand because the open landfills that are so important to the breeding and wintering distribution and population dynamics of this species, are due to close due to EU directives. The combination of landfill closures and rapid climatic change predicted for Iberia (Giorgi 2006, Perez and Boscolo 2010) makes the white stork particularly susceptible to future environmental change. Despite this, the impact of landfill use on productivity has never been quantified. In order to understand the consequences of environmental changes, individual data on movement behaviour was needed.

The white stork was an excellent model species to use for developing and trialling a new GPS/GSM data logger. This was done in conjunction with collaborators from the University of Lisbon. Two prototypes were trialled on the white storks, firstly a battery powered model, then a solar powered version (Figure 1.2).

The existence of migratory and non-migratory individuals in the same population provides an excellent opportunity to study the causes and consequences of different migratory decisions. However, data loggers are often not suitable for deployment on large numbers of individuals, especially low mass species or those with low survival or breeding site fidelity. Thus my thesis also tested whether stable isotopes could be used to reliably infer migratory status. Stable isotopes are not a suitable technique for white storks for two reasons. Firstly, their moult slows with age, meaning feathers are not replaced every year (van den Bossche *et al* 2002) and secondly, high intake of food from landfill sites means the isotopic signal is not linked to local diet or hydrology. Instead, the lesser kestrel was used as a model species.

The lesser kestrel also underwent rapid population declines in recent decades so was classified as globally Vulnerable (Birdlife International 2015). However recent evidence from the past 20 years suggests the population has stabilised or recovered slightly. This major causes of population decline are thought to be habitat loss due to agricultural intensification in both the breeding and wintering areas and, in Portugal, loss of nest sites (Forero *et al* 1996 do not consider lack of nest cavities as a limiting factor in the Spanish population). In Portugal the majority of the population now nests in artificial nest boxes rendering this species and the white stork both highly influenced by anthropogenic activities.

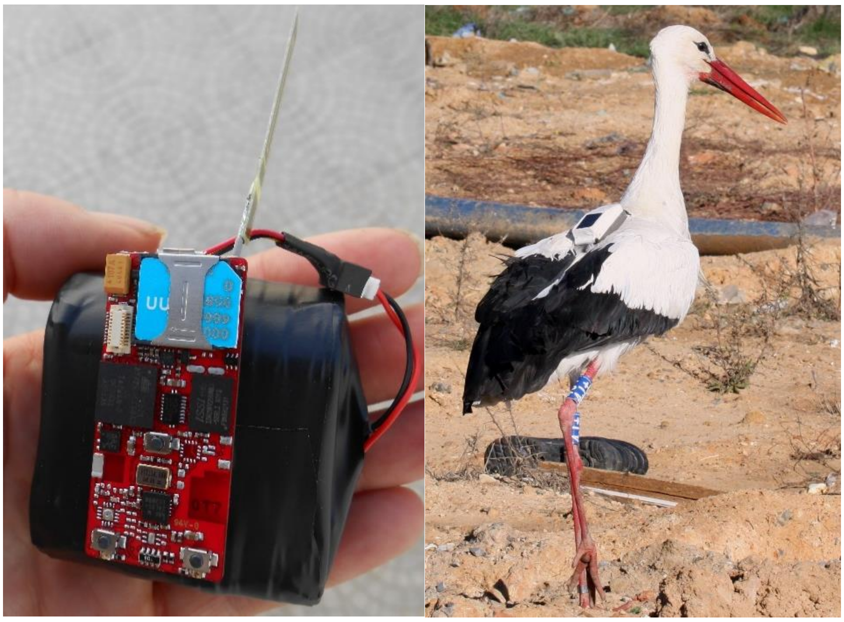

**Figure 1.2** *Battery powered (left) and solar powered (right) versions of the logger trialled as part of this study*.

### 1.3.2. Thesis aims and outline

This thesis can be split into two sections. In the first section (Chapters 2 – 5), the main objective was to provide insight into the consequences of artificial food resources on the breeding and winter season movement behaviour, foraging ecology and productivity of white storks. In the second section (Chapter 6), the aim was to test whether resident and migrant lesser kestrels could reliably be identified using stable isotopes from feathers.

Chapters are formatted as manuscripts with the objective of publishing each as an individual paper in peer-reviewed journals. Consequently, each chapter includes a list of references. Two chapters (Chapters 2 and 3) are currently in the editorial process for publication, the remaining chapters will be submitted for consideration in the near future.

This thesis consists of 5 data chapters, each addressing particular aims and questions:

**Chapter 2** assess the consequences of landfill use on the large-scale spatial and temporal movement patterns of resident white storks throughout the year using GPS tag data and answers questions including:

i) How does the extent and consistency of landfill use compare during the breeding and non-breeding seasons?
ii) How does landfill use impact on nest use, daily distance moved and foraging ranges?

**Chapter 3** explores the climatic drivers behind winter landfill use and asks:

i) What weather conditions influence winter foraging behaviour?
ii) How may future climatic change impact resident white stork populations?

**Chapter 4** investigates variation in breeding success as a consequence of relative use of landfill and non-landfill habitats. In view of imminent landfill closure, it is important to quantify the current impact of landfill use on productivity and to assess how this may change, so the main questions asked in this chapter are:

i) What is the impact on productivity of colony-landfill distance and distance from the colony to the most utilized non-landfill habitat?

**Chapter 5** tests a hypothesis raised in Chapter 4 and analyses the impact of foraging habitat on the heavy metal content of egg shells.

i) Are levels of selected heavy metals higher in colonies close to rice field habitats, compared to colonies close to landfills or control habitats?

**Chapter 6** is a stable isotope analysis from feathers of lesser kestrels with the aim of determining if it is possible to distinguish birds of differing migratory status (resident or migrant). As feather deuterium values were discovered to be unexpectedly enriched, another aspect of this chapter became an investigation into the interpretation of enriched deuterium. Thus the research questions were:

i) Which isotope, or combination of isotopes, best distinguishes Iberia and the Sahel, and can they be used to reliably assign individual migratory status?
ii) Can differences in deuterium enrichment between age classes be used to identify juvenile (non-breeding) birds?

**Chapter 7** brings together the findings of other chapters to discuss how information acquired by this thesis may be useful in managing the impact of landfill closures on white storks. I make suggestions for future research directions that will continue to enhance our understanding of the movement and behaviour of species in response to future predicted climate and environmental changes.

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# Chapter 2

## Are white storks addicted to junk food? Impacts of landfill use on the movement and behaviour of resident white storks (*Ciconia ciconia*) from a partially migratory population.

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## Abstract

The migratory patterns of animals are changing in response to global environmental change with many species forming resident populations in areas where they were once migratory. The white stork *Ciconia ciconia* was wholly migratory in Europe but recently warmer European winter temperatures and guaranteed, year-round food from landfill sites has facilitated the establishment of resident populations in Iberia. In this study 48 resident white storks were fitted with GPS/GSM data loggers (including accelerometer) and tracked for 9.1 ± 3.7 months to quantify the extent and consistency of landfill attendance by individuals during the non-breeding and breeding seasons and to assess the influence of landfill use on daily distances travelled, percentage of GPS fixes spent foraging and non-landfill foraging ranges.

Resident white storks used landfill more during non-breeding (20.1% ± 2.3 of foraging GPS fixes) than during breeding (14.9% ± 2.2). Landfill attendance declined with increasing distance between nest and landfill in both seasons. During non-breeding a large percentage of GPS fixes occurred on the nest throughout the day (27% ± 3.0 of fixes) in the majority of tagged storks. This study provides first confirmation of year-round nest use by resident white storks. The percentage of GPS fixes on the nest was not influenced by the distance between nest and the landfill site. Storks travelled up to 48.2 km to visit landfills during non-breeding and a maximum of 28.1 km during breeding, notably further than previous estimates. Storks nesting close to landfill sites used landfill more and had smaller foraging ranges in non-landfill habitat indicating higher reliance on landfill. The majority of non-landfill foraging occurred around the nest and long distance trips were made specifically to visit landfill.

The continuous availability of food resources on landfill has facilitated year-round nest use in white storks and is influencing their home ranges and movement behaviour. White storks rely on landfill sites for foraging especially during the non-breeding season when other food resources are scarcer and this artificial food supplementation probably facilitated the establishment of resident populations. The closure of landfills, as required by EU Landfill Directives, will likely cause dramatic impacts on white stork populations.

## 2.1. Introduction

The migratory patterns of animals are changing in response to global environmental change (Walther *et al* 2002, Cotton 2003, Both 2009). Many previously wholly migratory bird species that used to winter in sub-Saharan Africa are forming resident populations in their southern European breeding grounds (Atlas Team 2008). The migratory strategy an individual adopts may impact on subsequent survival and lead to different population dynamics between migrant and resident individuals. Even small differences in survival and productivity associated with migratory strategy may lead to very rapid changes in the proportion of the overall population that migrates. Whilst migrants undergo energy demanding large-scale movements, residents are able to occupy the best breeding areas. Resident birds are known to breed earlier than migrants and have larger clutches (Massemin-Challet *et al* 2006). Due to the seasonal decline in food supply, early nests are known to have higher breeding success (Tryjanowski and Sparks 2008). However, residents usually experience less favourable environmental conditions in the breeding areas during the winter, affecting their survival directly or indirectly through food availability (Newton 1998, Newton 2007). The ability of resident birds to find food resources during this period may therefore be key for their survival. The ecology of migratory species that have become resident is not well understood. In particular, the movement behaviour of resident populations in the non-breeding season is poorly studied. Understanding the role of food availability in driving changes in resident bird distribution and movement behaviour will improve our ability to predict how partial migratory species may respond to future climate and environmental change and assist in designing effective conservation strategies.

Food-supplementation in birds has been shown to advance bird phenology [Chamberlain *et al* 2009, affect singing behaviour (Saggese *et al* 2011) and increase fledging success (Hilgartner *et al* 2014) but there is little knowledge on the impacts of providing constant and reliable artificial food resources on the movement patterns and migratory behaviour of birds.

Artificial food available from landfill sites may have facilitated the recent establishment (since the 1980s) of resident white stork populations in Iberia (Gordo *et al* 2007). This is within the lifetimes of individual birds in this long-lived (~25 years), iconic species. The causes of these changes in behaviour are not fully established but milder European winter temperatures due to climatic change (Massemin-Challet *et al* 2006), increased winter food availability from landfill sites (Gordo and Sanz 2006) and foraging on the invasive red swamp crayfish *Procambarus clarkii* in rice fields (Rosa 2005) have been proposed as likely factors. Foraging on landfill is undoubtedly a major influence as 80% of overwintering white storks in Iberia congregate near landfill sites (Tortosa *et al* 2002) and landfill forms 68.8% of local diets in both adults and juveniles throughout the year (Peris 2003). White storks also preferentially nest near landfill sites (Tortosa *et al* 2002) which has consequences for population distribution and range expansion patterns. Foraging on landfill is also a relatively new occurrence in the Eastern Europe stork populations (Kruszyk and Ciach 2010).

The number of overwintering white storks in Portugal has increased dramatically in recent decades (from 1,187 individuals in 1995 to 10,020 in 2008 (Rosa *et al* 2009) and to approximately 14,000 birds in 2014 (Rosa, personal communication)), simultaneously, the number of migrant individuals crossing the Straits of Gibraltar has increased by 86.4% between 1985 and 2004 (Nevoux *et al* 2008) and recent data indicates this trend continues (Onrubia *et al* 2014) suggesting that the overall population is increasing, not simply changing in migratory behaviour.

This study is the first to assess the consequences of reliable and abundant food resources (landfill sites) on the large-scale movement patterns of a recently established resident population of a previously wholly migratory species. We assess the spatial and temporal changes in movement behaviour throughout the year using newly developed GPS/GSM technology. We quantify the extent and consistency of landfill use by resident individuals during the breeding and non-breeding seasons and assess its influence on nest use, daily travel, foraging and non-landfill foraging ranges.

## 2.2. Methods

### 2.2.1. Study area and study system

Data loggers were deployed on 48 birds captured on active landfill sites during the winters of 2012/13 (*n=15*) and 2013/14 (*n=33*). Licenses to catch and deploy loggers were granted by the Instituto da Conservação da Natureza e das Florestas (ICNF). Five landfill sites across south-central Portugal were used: Aterro Sanitário de Ermidas do Sado (38.021444, -8.353319, *n=11*), Aterro Sanitário de Vila Ruiva (38.243040, -7.952321, *n=10*), Aterro Sanitário Intermunicipal de Évora (38.538004, -7.971274, *n=16*), Aterro Sanitário da Herdade do Montinho, Beja (37.924916, -7.864950, *n=8*) and Aterro Sanitário do Barlavento, Portimão (37.214041, -8.522350, *n=3*). Birds nested a maximum of 48.2Km from their capture location. The surrounding habitat was largely Mediterranean cork oak woodland (montado), a traditional low intensity management system consisting of savannah-like grassland with Cork oak *Quercus suber* and holm oak *Quercus rotundifolia* trees in varying densities used for cattle grazing and low intensity agriculture. The surrounding area also included non-irrigated agriculture, often in multi-annual crop rotation cycles, irrigated agriculture, rice fields and small plantations of olive trees and of deciduous and evergreen forestry. Urban settlements were mostly low density, apart from the city of Évora (population 56, 600).

Storks were captured using nylon and rubber leg lassos and a remotely activated, baited clap net. Both were deployed on the actively worked landfill and monitored continuously. Birds were detained for maximum of half an hour after capture. They were colour ringed on each leg and sex was estimated at time of capture from physical characteristics (body size, ruff size and bill length), a method known to be correct in 89% of cases (Ćwiertnia *et al* 2006)). In this study, sex was subsequently confirmed as correct in all six birds who were observed copulating.

There appeared to be no adverse effects of the deployment process. Several individuals were resighted in the days immediately following logger deployment and were behaving normally. Capture dates, total tracking time and the number of days of data available for each season are listed in Table 2.1.

Nests of tagged birds were easily detected as a location of tightly clustered GPS coordinates in continuous use. These locations were visited to confirm nest occupancy. All tagged bird nests were visited on 2-3 occasions throughout the breeding season (March and late May/early June) to monitor breeding parameters. Nests were observed with a telescope and, where possible, a camera pole was used to look in to the nest on each visit to assess clutch size and chick age (based on visual assessment of bill length and plumage development).

**Table 2.1** *Sex, tracking dates and number of days of tag data for each white stork. Breeding onset from accelerometer data (A) and/or field observations (F).*

| Sex* | Capture Date | End of Breeding | No. Days of Data | | Total | Breeding Onset Verification | Date Last Tracked |
|---|---|---|---|---|---|---|---|
| | | | Non-breeding | Breeding | | | |
| M | 15/11/2012 | 06/06/2013 | 118 | 85 | 203 | A + F | 14/02/2014 |
| (F) | 15/11/2012 | 28/05/2013 | 123 | 71 | 194 | A | 13/05/2014 |
| F | 16/11/2012 | 21/06/2013 | 112 | 105 | 217 | A + F | 16/09/2013 |
| (F) | 24/11/2012 | 04/06/2013 | 119 | 73 | 192 | A | 11/01/2014 |
| (M) | 24/11/2012 | 30/03/2013 | 126 | 62 | 188 | A + F | 05/06/2013 |
| (F) | 25/11/2012 | 17/04/2013 | 101 | 42 | 143 | A | 21/07/2013 |
| (M) | 25/11/2012 | 19/05/2013 | 113 | 62 | 175 | A + F | 24/06/2013 |
| (F) | 30/12/2012 | 08/06/2013 | 78 | 82 | 160 | A + F | 24/09/2013 |
| (F) | 30/12/2012 | 02/05/2013 | 99 | 24 | 123 | A | 15/09/2013 |
| (M) | 17/01/2013 | 31/05/2013 | 66 | 68 | 134 | A | 12/09/2013 |
| (F) | 17/01/2013 | 12/06/2013 | 68 | 78 | 146 | A | 12/06/2013 |
| F | 29/11/2013 | 06/06/2014 | 90 | 99 | 189 | A + F | 10/10/2014 |
| F | 08/12/2013 | 10/06/2014 | 88 | 96 | 184 | A + F | 11/11/2014 |
| M | 11/12/2013 | 01/06/2014 | 82 | 90 | 172 | A | 28/06/2014 |
| (M) | 11/12/2013 | 01/06/2014 | 83 | 89 | 172 | A + F | 26/06/2014 |
| (M) | 15/01/2014 | 01/06/2014 | 83 | 89 | 137 | A | 21/08/2014 |
| F | 02/02/2014 | 17/04/2014 | 50 | 24 | 74 | A + F | 01/06/2014 |

**Sex: in brackets was estimated at time of capture from physical characteristics, without brackets was confirmed from copulation*

### 2.2.2. Data loggers and the identification of behaviours

Newly developed GPS-ACC data loggers, developed by our team, were used in this study. All loggers were back-mounted on a teflon harness with biodegradable stitching to prevent lifelong placement. Loggers weighed 90g (battery powered, less than 4% of the total mass of the bird) and 45g (solar powered). After deployment, loggers quickly sank below the feathers minimising drag.

Loggers were programmed to wake up 5 times per day at 6am, 9am, 12am, 3pm and 6pm GMT. Each wake up (termed a data burst) obtained 10-20 consecutive GPS fixes (±20 m accuracy) and 3D accelerometer readings (at 1Hz with a sensitivity of ±6 G), once per second. Data automatically transmitted via GPRS using the GSM mobile phone network every 2 days to a web platform.

The information obtained from the accelerometer axes X (surge), Y (sway) and Z (heave in gravity) plus speed (derived from GPS positions) were used to determine behaviour during each data burst. Assigning a behaviour to each data burst allowed non-foraging behaviour (flight, inactive) to be excluded from foraging analysis, it was not to derive time budgets. The information of each variable was summarized by calculating the mean, standard deviation, max, min and range for the the whole duration of each data burst. Subsequently, each data burst was classified into four behaviour categories: inactive (standing and/or preening), foraging, flight and tending eggs. In the rare occasion that multiple behaviours were captured in a single data burst, the behaviour occurring at the end of the data burst was used. The behaviour associated with each data burst was then assigned to a GPS location derived from the end of the transmission (termed a GPS fix). This was because GPS position was fixed most accurately later in the transmission. Initially, the behaviours in a set of 500 randomly selected data bursts were manually classified for 6 birds based on field observations and, where there was ambiguity, reconstructions of accelerometer output in real-time with a hand held accelerometer linked to a computer, also analysing with pictures and video footage the position of the logger on the birds back. These behaviours were split into two independent sets: 400 randomly selected behaviours were used for training and 100 for validation of a classification tree model of the four behaviour classes (10 rounds). Behaviour classification analysis was done with R using the *rpart* library and the final classification-tree model was selected based on the lowest training data cross-validation error. The overall model accuracy was assessed using a multi-class AUC test (HandTill 2001 library (Hand and Till 2001)), resulting in a single AUC value of 0.97, indicating a good level of classification of the four behaviours. Example graphs of each behaviour are shown in Figure 2.1. Individual classification performance can be found in the confusion matrix (Appendix 2.1).

### 2.2.3. Determination of onset and end of breeding

For the purpose of this study, the non-breeding season is defined as the period between capture (November–February) and initiation of breeding detected in the tri-axial accelerometer data and verified with field observations.

The accelerometer data enabled the identification of the initiation of breeding designated *"*tending eggs*"*, characterised by birds looking down into the nest from a standing position. Our model predicted tending eggs behaviour with 87.0% accuracy (Appendix 2.1). This characteristic body position is observed in the field during the early breeding season and is a good indication that eggs are present. Tending eggs only occurred for a period of between 4 – 5 weeks during the incubation phase, only occurred in GPS fixes on the nest, and was detected in all breeding birds. The onset of this behaviour in the accelerometer data is abrupt and distinctive and matched observed timings for egg laying in all tagged birds with confirmed field data (n=9). The error between the appearance of the tending eggs behaviour in the accelerometry data and field observations of the presence of eggs was ±1 day. For the remaining birds (n=8), egg lay date is based on accelerometry and hatch date was also estimated based on chick ages observed by telescope (Table 2.1).

The breeding season is defined as the period from the initiation of breeding until either (1) breeding failure (n=6), (2) chicks fledged (n=8) or (3) the logger stopped transmitting (n=3). Failed breeders abruptly abandoned the nest for a period of 3-5 days. Fledge date was known precisely in 5 successful nests because chicks were tagged with GPS devices (n=2 nests) or chicks were seen fledging (n=3 nests). The remaining 3 nests were monitored and fledge date was estimated from chick age and development during late season colony visits. After fledgling chicks usually remain in the vicinity of the nest and continue to be supplementary fed by the adults for a period of one or two weeks, so for all nests fledgling date was defined as the earliest possible fledge date.

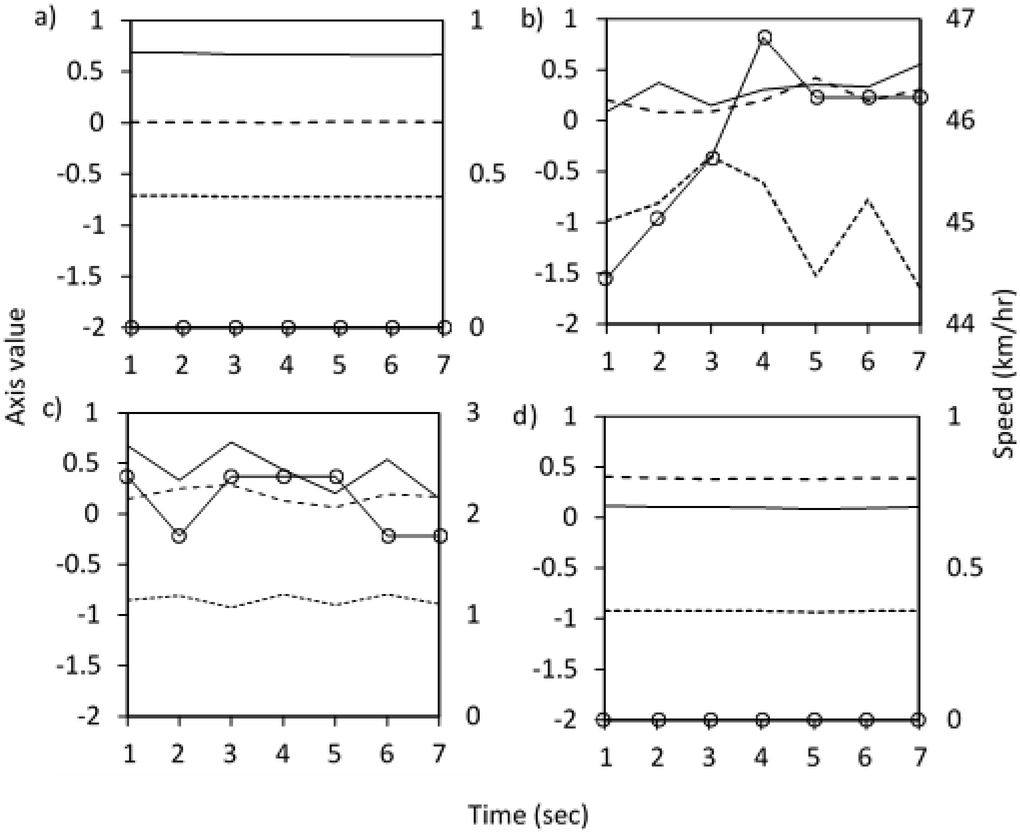


**Figure 2.1** *Examples of the 4 behaviour classes used to train the model. White stork behaviours identified using the accelerometer data: a) inactive (standing), b) flight, c) foraging, d) tending eggs. The three axes and speed are represented, X axis- surge (black line), y axis -sway (dashed line), z axis -heave (dotted line) and speed (line with circles).*

This analysis focuses exclusively on the behaviour of breeding birds during the pre-breeding and breeding period, no post breeding data are included. Similarly, juveniles (n=3) and non-breeding birds (n=5) were also omitted. Juveniles were distinctive due to their dispersive nature and lack of obvious cluster of GPS fixes which would have marked the location of a nest. Other non-breeding birds were associated with a nest but displayed no breeding behaviour as determined from accelerometer data (no tending eggs behaviour) and field observations.

One tagged individual died prior to breeding. Twenty-two loggers stopped transmitting prior to or shortly into the breeding season and therefore could only be included for consideration of maximum distance travelled between nest and landfill. Here we present results from 17 tagged breeding birds, *n=7* assessed to be male and *n=10* assessed to be female (Table 2.1).

### 2.2.4. Non-breeding and breeding season landfill use

GPS fixes from all data bursts (excluding fixes where the bird was in flight) were used to ascertain the percentage of fixes occurring on landfill, non-landfill habitat and within 20 m of the nest. This included the 6am data burst, despite it occurring before sunrise during winter and well before conditions favourable for thermals suitable for long flights. White storks are known to forage on moonlit nights and were regularly observed leaving their nest on or before first light. Sensitivity analysis of a subset of tagged birds revealed that patterns of winter landfill use were highly variable between individuals (Appendix 2.2) with an indication that birds nesting further from landfill were more likely to use landfills at 6am than birds close by. In one individual during the non-breeding period, 8% of GPS fixes on landfill occurred at 6am. Such visits may make a crucial contribution to winter survival probability.

A 50 m buffer was drawn around each landfill and all points within this buffer were considered as landfill. This 50 m buffer captured occasions where birds were disturbed and temporarily flushed off the landfill to just beyond the site perimeter. Distance from the nest to landfill was determined using the minimum straight-line distance between the nest and the centre of the utilised landfill site. GPS fixes within

20m of the nest were removed from foraging analyses and analysis of seasonal reliance on landfill because on the nest the birds are usually inactive.

### 2.2.5. Daily distance

Non-breeding and breeding season mean daily distance travelled was calculated using GPS locations only from days where all 5 data bursts were available (including fixes in flight). This varied between individuals from 34.2% to 98.8% of total data bursts (mean ±SE non-breeding: 77.6 ± 3.7, breeding: 68.2 ± 4.9). The distance between successive GPS positions (pairs of latitudes and longitudes) were calculated in kilometres then summed to obtain a daily totals. Daily totals were then used to create a non-breeding and breeding season mean for each individual.

### 2.2.6. Foraging behaviour and home range estimates

Foraging range was derived by calculating the 50% and 95% utilization distribution kernels for each bird using only data bursts where accelerometer information indicated the bird was foraging. Kernel polygons were determined with the R library *ade-habitat* and imported into ArcGIS to calculate kernel area. Data bursts where the individual was standing, flying or on the nest were excluded. The aim of this analysis was to investigate natural foraging habits so data bursts occurring within 50m of landfill sites were also removed.

## 2.3. Statistical Analysis

Data are normally distributed so paired t-tests were used to assess seasonal differences in the percentage of GPS fixes spent by the nest, on landfill and in non-landfill habitat. Linear regressions were used to explore the influence of distance from nest to land fill site on seasonal landfill use in the breeding and non-breeding seasons and to determine the relationship between nest-landfill distance and the percentage of data bursts assigned as foraging behaviour. Paired t-tests and non-linear regression were used to compare differences in mean travel distances between seasons. Foraging range size (50% and 95% kernels) were log transformed for normality and linear-regression was used to test the relationship between range size with nest-landfill distance.

### 2.4. Results

48 birds were tracked for a total of 155 months (mean per individual 9.1 ± 3.7 months, mean data bursts per day: 4.17 +/- 0.15). This study focuses on 10,425 data bursts (613.2 ± 41.3) from 17 birds, 5758 during the non-breeding season (338.7 ± 26.6) and 4667 during breeding (274.5 ± 23.2).

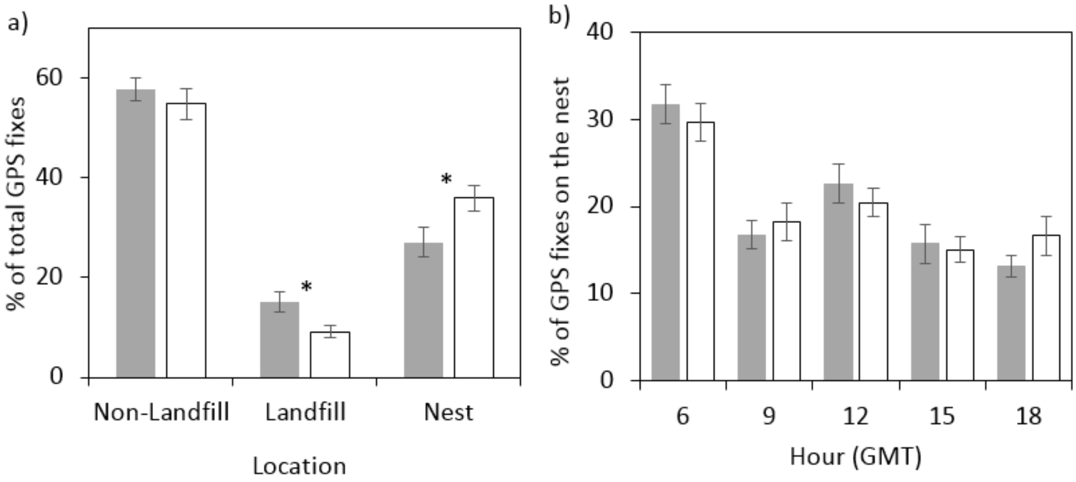


**Figure 2.2** *White stork habitat and nest use during the non-breeding (filled bars) and breeding (open bars) seasons. a) Seasonal differences in percentage of total GPS fixes (±SE, excluding flight) registered in non-landfill habitat (t(16)=1.465* p= *0.162), landfill sites (t(16)= 2.63,* p= *0.018) and on the nest (t(16)= -4.36,* p= *<0.001). b) Frequency of GPS fixes occurring on the nest during each of the 5 daily data bursts as a percentage (±SE) of all transmissions within 20m of the nest. Asterisks indicate statistically significant differences.*

### 2.4.1 Seasonal foraging habitat, landfill and nest use

The percentage of GPS fixes on non-landfill habitat was similar in the breeding and non-breeding seasons (Figure 2.2a, paired t-test, t(16)=1.465, p=0.162).

During the non-breeding season, a large percentage of total GPS fixes were spent on the nest (mean 27.1% ± 2.97) with 25% of the birds (assessed as both males and females) spending up to 49.7% of GPS fixes within 20 metres of the nest. Individuals of both sexes were found on their nests throughout the day and there was no significant difference in the hour of nest attendance between seasons (Figure 2.2b). 22.6% ± 2.24 of non-breeding GPS fixes on the nest occurring at midday (Figure 2.2b), indicating nest use throughout the day, not just as a night roost. The percentage of GPS fixes on the nest was not related to the distance between nest and landfill site during either the breeding F(1,15)=0.011, p= 0.915) or non-breeding F(1,15)=0.035, p= 0.855) seasons. Behavioural data, derived from accelerometery, indicated that in the majority of fixes on the nest the birds are inactive (inactive fixes within 20m of the nest: 87.2% ± 6.4 non-breeding, 86.9% ± 6.4 breeding). Post breeding data from 8 individuals tracked until at least September (Table 2.1) indicated that all birds continued to remain on their nests after chicks fledged.

All 17 individuals that were tracked during non-breeding and breeding seasons used landfill to some extent in the non-breeding period, whilst one individual did not use landfill at all during breeding and a second only rarely (0.7% of data bursts). Although there were individual differences, overall percentage of fixes on landfill was significantly higher during the non-breeding season (mean ± SE 20.1% ± 2.3 of GPS fixes) compared to the breeding season (14.9% ± 2.2), (paired t-test, t(16)=2.63, p= 0.018, Figure 2.2a). 35.3% (*n=6*) of individuals had higher attendance on landfill during the non-breeding season, the majority of individuals 52.9% (n=9) used landfill approximately equally in both seasons and two birds used landfill more during breeding (Figure 2.3).

### 2.4.2. Impact of distance between nest and landfill on landfill use

Regardless of differences in landfill use between seasons, individuals closer to landfill used this resource more frequently than their more distant conspecifics and landfill attendance declined with increasing straight-line distance between the nest and landfill site in both seasons (Figure 2.4 a and b, non-breeding $R^2$=0.257, p= 0.045, breeding: $R^2$=0.414, p= 0.007). Distance from nest to landfill is strongly correlated with frequency of landfill use during breeding (Figure 2.4b).

One white stork had a different strategy compared to all other birds in this study (unfilled square symbol, Figures 2.4 and 2.6). This individual was frequently detected on landfill during breeding, despite having the largest nest-landfill distance (28.1 km). This was probably due to a lack of non-landfill resources in the vicinity of the nest during breeding, likely associated with the local timing of rice field drainage. This bird was considered an outlier, and was not included in the analyses.

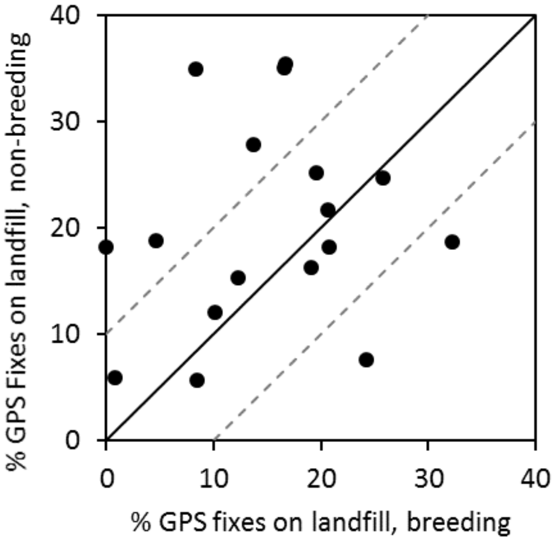


**Figure 2.3** *Frequency of GPS fixes away from the nest (excluding flight) occurring on landfill sites in the breeding and non-breeding seasons for 17 white storks. Dashed lines are the 10% intervals around the line that represents equal use of landfill in both the non-breeding and breeding seasons. 6 birds use landfill less in the breeding season (points above dashed line), 9 individuals use landfill equally in both seasons (points inside dashed lines), and 2 birds use landfill more in the breeding season (points below dashed lines).*

During non-breeding, white storks travelled further to visit landfill sites, with almost one quarter (23.5%) of tagged birds travelling from ≥25 km to reach landfill, whereas in the breeding season the maximum distance travelled was 28.1 km. We found a positive quadratic response, particularly during breeding (Figure 2.4d), with the mean distance travelled increasing with distance to the nest until approximately 14-15 km and then as the nest-landfill distance increased further, the mean distance travelled decreased. This suggests a threshold distance that birds will preferentially travel to landfill. Birds with nests located at this distance travelled larger daily distances (travelling further to visit landfill sites) than birds close to landfill or further away. Birds travelled further during the breeding season (mean ±SE 11.19km ± 1.46 per day) compared with the non-breeding season (7.91km ± 0.69, paired t(16)= -2.37**,** p= 0.031).

### 2.4.3. Foraging behaviour and foraging range

The percentage of GPS fixes away from the nest assigned as foraging behaviour increased from 45.5% ± 2.7 during non-breeding to 57.5% ± 3.1 during breeding (paired t(16)=-3.667, p= 0.002). During breeding birds nesting further from landfill spent a higher percentage of GPS fixes in foraging behaviour (Figure 2.5a and b). While during the non-breeding season there was no effect of distance to landfill on the percentage of foraging GPS fixes. All birds, except one, marked as a triangle, fitted this pattern. Analysis including this individual found no relationship in either the non-breeding ($R^2$= 0.001, p= 0.919, mean Mahalanobis distance ± SD: 0.941 ± 1.017) or breeding season ($R^2$= 0.037, p= 0.461, Mahal: 0.941 ± 0.913).

During the breeding season, white storks increased their foraging range (measured by kernel 50% and 95%) with increasing distance between the nest and the landfill site (50% kernel: F(1,14)= 7.225, $R^2$= 0.340, p= 0.018, 95% Kernel: F(1,14)= 5.270, p= 0.38, $R^2$=0.273, Figure 2.6). There was no significant increase in foraging range with distance between nest and landfill during non-breeding (50% kernel: (F(1,14)= 0.19, $R^2$= 0.013, p= 0.67, 95% Kernel: F(1,14)= 0.130, $R^2$= 0.009, p= 0.72).

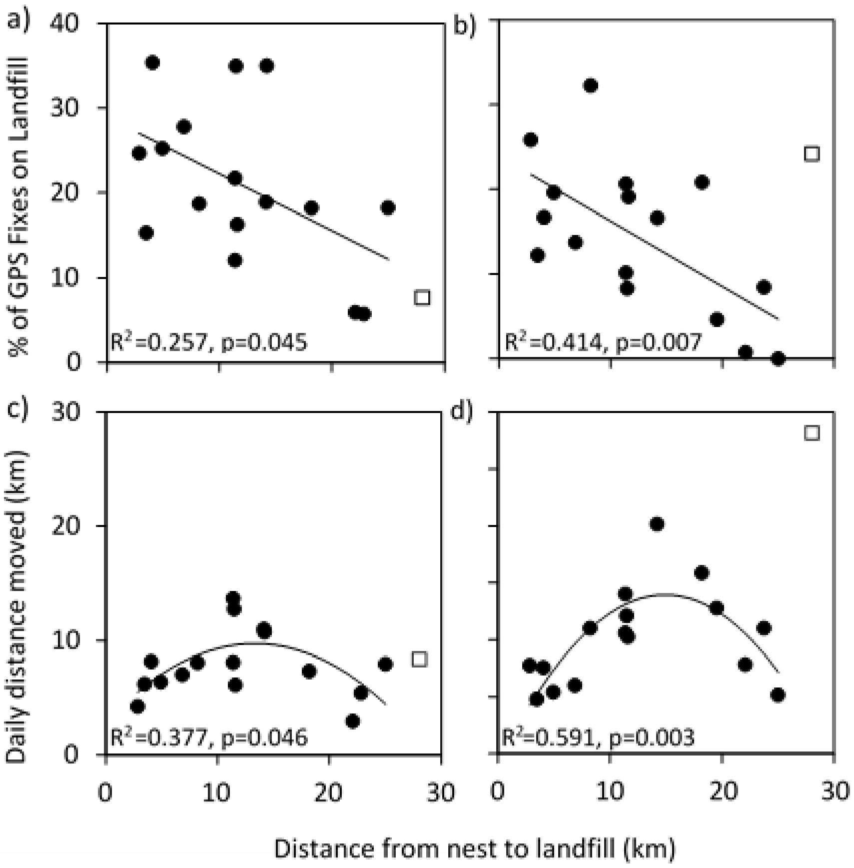


**Figure 2.4** *Percentage of GPS fixes on landfill (excluding fixes in flight and within 20m of the nest) in relation to distance from nest to the landfill site during a) non-breeding and b) breeding seasons. Total daily distance moved (derived from all available fixes, including flight and nest) in relation to distance between the nest and the landfill site during c) non-breeding and d) the breeding season. One individual was considered an outlier (unfilled square) and was excluded from the linear regressions, see results section.*

## 2.5. Discussion

### 2.5.1. Winter nest use

This study provides first confirmation of year round nest use, an entirely new behaviour that has developed as the Iberian population of white storks shifted from being wholly migratory to partially migrant. There is no evidence from previous monitoring studies (Blanco 1996, Massemin-Challet *et al* 2006, Archaux *et al* 2008) to suggest ringed birds occupied their nests all year, perhaps because year-round nest use is a recent phenomenon. Data from tracking studies of migratory white storks indicate that, whilst highly faithful to their breeding grounds, individuals have little wintering site fidelity and pairs do not winter together (Berthold *et al* 2002). Landfill sites provide abundant food resources that are reliable in both space and time, thus likely contributing to enabling individuals to remain in their breeding territory and on their nests year-round. This is extremely rare in temperate zones because, during winter resident individuals of other species usually perform regional or local movements away from their breeding territory and/or form loose flocks that are highly mobile to track limited, dynamic winter food resources (Newton 1998).

Nest use and maintenance was observed throughout the day during the non-breeding season (Figure 2.2b) with both males and females spending up to 49.7% of GPS fixes within 20 metres of the nest. This suggests the nest is defended during the winter rather than simply being used as a roost site at night. Field observations confirmed it is not uncommon to see pairs on the nest throughout the day engaged in nest defending and repair (authors, personal observations) during the non-breeding season. Whilst in the vicinity of the nest birds are inactive (standing/preening) rather than engaged in foraging behaviour, which is a significant time investment during winter when daylight foraging hours are shorter. All individuals displayed this behaviour independent of distance between the nest and the landfill site.

Nests near guaranteed food supply from landfill are highly desirable locations (Tortosa *et al* 2002, Peris 2003**,** Kruszyk and Ciach 2010) and therefore it was predicted these would require more defending than nests in non-landfill locations. However, the lack of correlation between the percentage of GPS fixes on the nest and either distance between nest and landfill or frequency of landfill attendance indicates

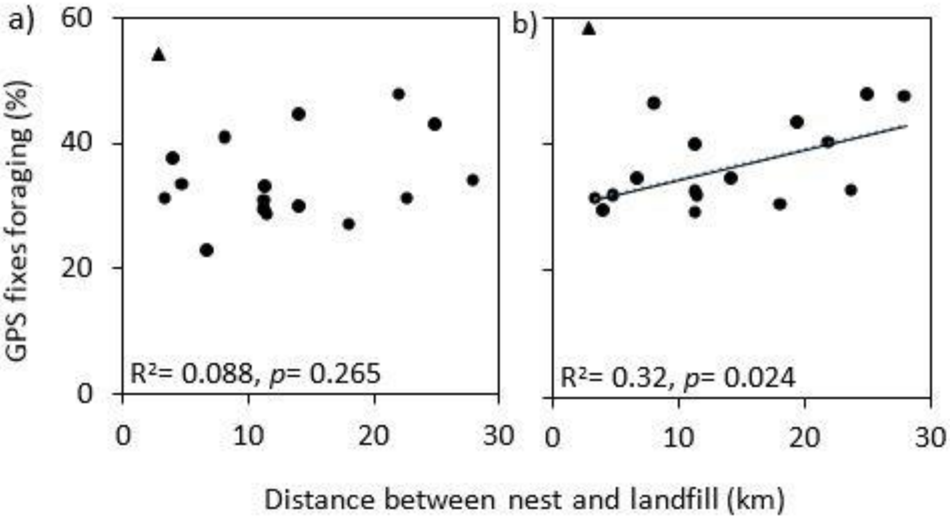


**Figure 2.5** *Foraging behaviour in relation to nest-landfill distance. Percentage of total foraging behaviour GPS fixes (defined from all GPS fixes) in relation to distance from nest to landfill during the a) non-breeding and b) breeding seasons. One individual (triangle) had an exceptionally high percentage of foraging behaviour GPS fixes during both the non-breeding and breeding seasons and was excluded from the linear regressions presented in this figure. Analysis including this individual showed no significant relationship.*

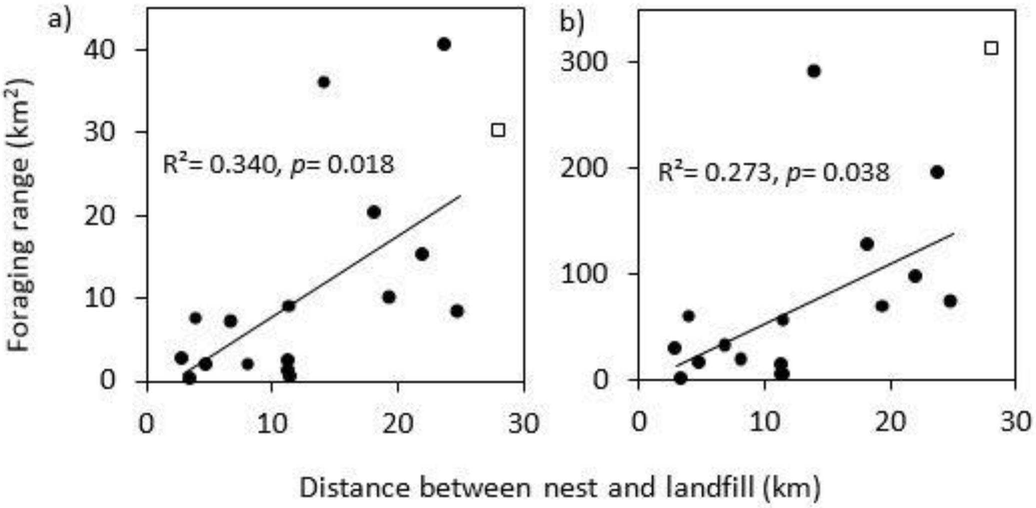


**Figure 2.6** *Breeding season foraging ranges in non-landfill habitat with distance from nest to landfill. a) 50% and b) 95% UD Kernels constructed from foraging GPS fixes. One individual (unfilled square) is excluded from the linear regressions (see results section).*

individuals defend their nest regardless of proximity to landfill. This may be partially driven by other factors including proximity to high quality non-landfill habitat, colony size and the high white stork population density found in Iberia. It is unclear if white storks are limited by the availability of suitable nest locations around landfill sites. White storks nest in close proximity to each other on myriad structures from trees to pylons and other man-made constructs which suggests many nest options (Tortosa *et al* 2008) however, nest sites within specific colonies may be limited (Vergara *et al* 2010).

Residency enables an advance in breeding phenology and can increase the breeding success of residents compared to returning migrants in the same population, mainly due to earlier laying date (Massemin-Challet *et al* 2006). Nests near landfill fledge significantly more chicks (Tortosa *et al* 2002) and fledging success has been demonstrated to decline by 8% per kilometre distance from a supplemental feeding location (Hilgartner *et al* 2014). In populations of white stork that do not use landfill, arrival date is strongly correlated with fledgling success due to the seasonal decline in food availability (Kosiki *et al* 2004, Tryjanowski *et al* 2004). Abundant food from landfill sites therefore mitigates the seasonal decline in food availability.

### 2.5.2. Impact of nest distance on seasonal reliance on landfill

Overall, white storks were more reliant on landfill during the non-breeding season (Figure 2.2). All individuals in this analysis were caught on landfill sites so it was expected that all used landfill to some extent, however in the breeding season two birds (11%) did not use landfill. This study shows landfill site use varies considerably and is lower in Iberia than previously described (Peris 2003). Individuals nesting closer to landfill utilized this resource more frequently in both seasons than those nesting further away (Figure 2.4a and b) and landfill use declined with increasing distance between the nest and landfill, even during the non-breeding season, indicating that in both seasons distance from nest to landfill is the dominant factor determining reliance on landfill. During chick rearing, due to energy requirements and travelling time constraints, this relationship was expected. However, it was surprising during the non-breeding season, and may be because resident storks now also occupy their nests during the non-breeding season, rather than forming loose roaming winter flocks, thus foraging occurs from a central point, the nest, throughout the year.

The lower frequency of use of landfill during breeding is possibly due to prey size. Adult white storks may prefer to feed smaller food items foraged in non-landfill habitats to their chicks. White storks supplementary fed with large items (rats, small chickens, fish) had similar foraging rates to nests that were not supplementary fed until chicks were over 20 days old and able to handle larger items (Moritzi *et al* 2001). This is consistent with similar behaviour showed by gulls. Herring Gulls preferred soft, small foods (e.g. earthworms) in the first days after chick hatching; but immediately switched back to the more energetically profitable strategy of foraging on landfill as soon as chicks could swallow larger items (Pons 1994).

During the non-breeding season white storks travel larger distances to visit landfill sites. One in every four breeding birds analysed travelled over 25km and one bird travelled 48.2 km from its nest to the landfill during non-breeding, while in the breeding season the maximum distance travelled was 28.1 km. This revises previous work that suggest Iberian white storks travel 12 km to reach landfill (Tortosa *et al* 2002). Massemin-Challet *et al* (2006) defined non-landfill colonies as ones 15 km from landfill and Moritzi *et al* (2001) suggested storks travel an additional 4 km to reach supplemental food, both of which are under estimates for Iberian storks.

Distance from nest to landfill defines how far an individual is prepared to travel each day as well as how heavily landfill is used. The relationship is non-linear so daily distance moved increased with distance from the landfill whilst it remained beneficial (both energetically and in terms of leaving the nest undefended) to visit landfill (Figure 2.4 c and d). Thus, individuals who nest close to landfill use landfill more and travel lower daily distances. This effect is particularly strong during breeding when birds travel greater daily distances.

### 2.5.3. Foraging behaviour and foraging range

During non-breeding there was no significant effect of nest-landfill distance on the percentage of GPS fixes spent foraging (Figure 2.5a). This may be associated with seasonal changes in the quality of non-landfill habitat surrounding the nest, in particular the abundance of red swamp crayfish. This important prey species is now prevalent in water ways across Iberia, particularly rice fields, and is more accessible to storks during winter when water levels are high (Almeida 2013). Crayfish abundance in the vicinity of the nest may therefore render the correlation between nest-landfill distance and percentage of GPS fixes spent in foraging activities less significant. During breeding, there is a general trend for birds close to landfill to have fewer foraging behaviour GPS fixes than those nesting further away (Figure 2.5b). The individual that did not follow this trend foraged extensively during both non-breeding and breeding, despite proximity to landfill (Figure 2.5), and is suspected to be a young, inexperienced bird breeding for the first time. Inclusion of this individual removed the significance of the relationship between foraging behaviour and nest-landfill distance, suggesting a greater sample size is required in order to fully capture the range of behavioural responses. During breeding the majority of foraging occurs close to the nest so individuals nesting close to landfill are more likely to visit landfill and the average distance at which it compensates to visit landfill decreases.

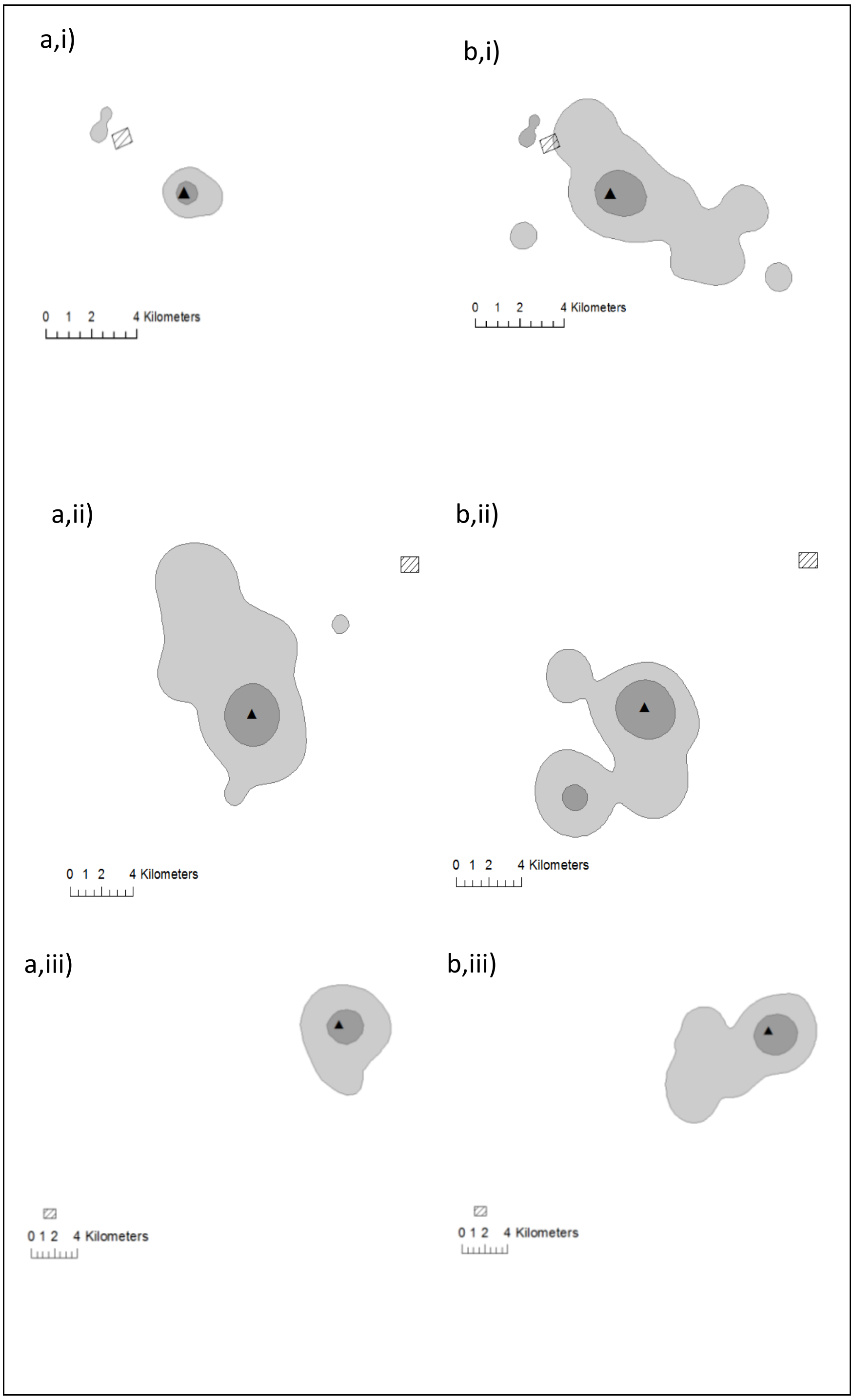
Foraging range in non-landfill habitat increased with distance from the nest to landfill site indicating that birds nesting further from landfill forage primarily in non-landfill habitat and require a larger foraging area. This was only significant during the breeding season (Figure 2.6). Kernel analysis indicated that across individuals and seasons, the majority of non-landfill foraging occurred immediately around the nest (Table 2.2), which is congruent with findings of previous studies (Ozgo and Bogucki, Alonso *et al* 1991, and Kosicki 2010). Landfill visits were usually specific, long distance excursions away from the nest that rarely included stops in non-landfill habitat *en route*. This may explain why distance from landfill had no effect on non-landfill foraging area during the non-breeding season. It also highlights the possibility for year-round depletion of local resources surrounding the nest.

The European Union Landfill Directive (1993/31/EC) set targets to progressively reduce the volume of biodegradable municipal waste entering landfills through to 2016 (PERSU II). As a result, open-air landfills are being replaced by covered waste processing facilities that are inaccessible to birds. In the immediate future there will be a sharp reduction in the availability of food waste that will have important consequences for Iberian white storks. This study is particularly relevant as it quantifies the extent to which the resident Iberian white stork population relies on artificial food, prior to the closure of landfill sites. It is highly likely that, despite following individuals caught on landfill only, this study can be applied to the majority of resident storks in Iberia. This is because most (80%, Tortosa *et al* 2002), if not all, overwintering birds are thought to use landfill sites to some extent, which represents a significant proportion of the non-breeding population, whilst during breeding, birds preferentially nest close to landfill (Tortosa *et al* 2002). Results presented here are therefore believed representative of the resident white stork population and provide a snap-shop of the current situation in terms of patterns of landfill use.

### 2.6. Conclusions

This study shows the effect of recent anthropogenic changes on the movement ecology and behaviour of a long-lived species through the provision of abundant and spatially stable food resources. This study presents robust evidence that resident white storks defend their nests year round and consequently spend a large percentage of GPS fixes attending the nest during the non-breeding season. The food resources, obtained on landfill sites, likely facilitated the establishment of resident individuals in a previously wholly migratory species. Frequency of landfill use by white storks decreases with increasing distance between the nest and the landfill, during both non-breeding and breeding seasons. During breeding birds nesting further from landfill spend proportionally more GPS fixes engaged in foraging behaviour and have larger foraging ranges in non-landfill habitat than birds nesting close to landfill sites. This will likely impact breeding success and population demography.

**Table 2.2** *Typical non-landfill foraging ranges for 3 individuals (i,ii,iii) nesting at varying distances from landfill. The non-breeding season a) and the breeding season b) are depicted. 50% (dark grey) and 90% (light grey) UD Kernels from foraging GPS fixes in non-landfill habitat. Nests were located at 2.9km (i), 11.5km (ii) and 25.0km (iii) from landfill. The shaded rectangle indicates the position of the landfill.*



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# Chapter 3

# The influence of weather conditions on foraging strategies and landfill use by white storks (*Ciconia ciconia*) overwintering in Southern Europe

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Gilbert, N.I., Silva, J.P., Pacheco, C., Correia, R.A., Catry, I., Atkinson, P. W., and Franco, A.M.A., The influence of weather conditions on foraging strategies and landfill use by white storks (*Ciconia ciconia*) overwintering in Southern Europe.

## Abstract

The white stork *Ciconia ciconia* was wholly migratory in Europe but in recent decades the number of individuals overwintering in Iberia has increased significantly. Overwintering is hypothesised to be a response to milder winter temperatures and increased food availability, including from anthropogenic sources such as landfill sites. Both changes in climate and in winter food availability likely contributed to population increases due to improved overwinter survival, yet little is known about the effect of winter climatic conditions on the relative use of landfill versus non-landfill habitats. Open landfills are scheduled to close by 2018 as a consequence of EU directives, so understanding the effects of these changes for overwintering birds is crucial.

This study uses information from newly developed, high precision GPS/GSM data loggers programmed to collect 5 GPS fixes per day, to model and assess the relative importance of winter daily climatic variables (precipitation, temperature, wind speed) and distance from roost to landfill on the use of landfill sites. Tags were deployed on 48 storks caught on landfill during the winters of 2012 and 2013.

Accumulated precipitation (10-day running mean) was the most significant predictor of landfill use (negative effect 11.5 %). This indicated that storks use landfill less during wetter periods, possibly due to higher availability of prey items in non-landfill habitats when conditions are more humid. Minimum temperature (5.5 %) significantly decreased the proportion of daily GPS fixes occurring on landfill, perhaps related to fewer thermals for soaring flight.

All storks utilized their nests throughout the winter. Consequently, the distance between nest and landfill was also an important predictor of landfill attendance (effect 8.0 %). White storks used their nest throughout the day and throughout the winter independently of climatic conditions.

Future climatic predictions for a drier, warmer regime will likely be problematic for the white stork, because it will decrease the suitability of winter non-landfill foraging habitat. It is likely that landfills currently buffer the wintering population against the impacts of dry winters.

## 3.1. Introduction

In response to global environmental change, the migratory behaviour of many species is changing (Walther *et al* 2002, Cotton *et al* 2003, Both *et al* 2009). Many avian species that used to be entirely migratory and winter in sub-Saharan Africa are now forming resident populations in their breeding grounds in southern Europe (Atlas Team 2008). For example, shifts in phenology, timing of migration and length of stay in the breeding area have been recorded in Iberia for many avian species including the iconic white stork (Gordo and Sanz 2006, Shephard *et al* 2015). In Portugal, the number of overwintering white storks has increased significantly from 1,187 individuals in 1995 to 10,020 in 2008 (Rosa *et al* 2009) and an estimated 14,000 individuals in 2014 (Rosa, personal communication). Increasing numbers of migrants from the Eastern European stork population now also overwinter in Iberia (NABU 2015). The drivers of these behavioural changes are not fully understood but both climatic and environmental factors are likely important. These include: i) milder European winter temperatures due to climatic change (Gordo and Sanz 2005, Massemin-Challet *et al* 2006) and ii) increased availability of winter food resources in the form of the invasive red swamp crayfish *Procambarus clarkii*, which is present in rice fields, and abundant, artificial food from landfill sites (Tortosa *et al* 2002, Rosa *et al* 2005, Sanz-Aguilar *et al* 2015).

Landfills are undoubtedly a major influence in determining white stork distribution during winter when it is estimated that 80% of wintering storks in Iberia congregate at landfills (Tortosa *et al* 2002). However, recent EU policies have instigated the ongoing, systematic closure of all open-air landfills in favour of covered recycling facilities which will be inaccessible to birds (Directive 2001/77/EC, PERSU II). This is likely to have important impacts, particularly on the survival of wintering white storks.

White storks are highly opportunistic and adaptable, but traditionally forage by wading in shallow water and wet meadows (Carrascal *et al* 1993). Most studies investigating the influence of winter climatic conditions on white storks refer to birds in their African wintering areas. Here, winter survival is correlated with rainfall, hence primary production (Kanyamibwa *et al* 1990, Schaub *et al* 2005). Precipitation is also important during breeding where it is correlated with reproductive success across the

European range (Saether *et al* 2006, Rosa *et al* 2005, Nevoux *et al* 2008). Whilst seasonal precipitation is important, white storks usually do not colonize areas that experience either high annual rainfall (Atlas Team 2008), or areas with long drought periods, hence their absence across much of central Asia (Lack 1966). Temperature is also important because white storks avoid cool areas that experience significant frost (Cramp and Simmons 1977). For example, Saether *et al* (2006) noticed a population increase after a warm pre-breeding season, probably related to improved food availability.

Thus, so far, the warmer winter temperatures experienced in Iberia seem to have been favourable for storks, enabling an overwintering strategy to develop (Gordo and Sanz 2006, Pulido and Berthold 2010, Shephard *et al* 2015). However, the Mediterranean is a climate change hot-spot (IPCC 5AR, 2013), and one of the world's most responsive regions to climate change (Giorgi 2006). Little is known about the winter ecology of resident white storks, particularly the climatic drivers behind landfill use, or how future climate change predictions for Iberia may impact overwintering birds. White storks are particularly vulnerable to potentially detrimental environment change in the near future due to the combination of landfill closures and rapid climatic change. It is therefore essential to identify the role of weather conditions in influencing winter foraging behaviour and determine how future climatic change may impact resident Iberian white stork populations. The aims of this study are 1) to quantify the current importance of landfill sites for the white stork and 2) to determine which climatic and physical factors predict winter landfill use.

## 3.2. Methods

### 3.2.1. Study area

This study was conducted in the Alentejo region of south Portugal. This area consists of flat plains and low, rolling hills. The climate is classified as Hot Summer Mediterranean (Peel *et al* 2007). Winters are relatively mild and wet with a January mean of 5-8$^0$C and most (75%) of annual rainfall (500 - 600mm) usually occurs between October and March (Moreira *et al* 2005). Summers are hot and dry, the Alentejo being one of the hottest regions in Europe with regular summer temperatures of $40^0$C (mean $30\text{-}35^0$C).

The non-landfill habitat in the Alentejo largely consists of Mediterranean cork oak woodland landscape (called montado in Portugal, or dehesa in Spain), a traditional low intensity agro-forestry system of cork oak *Quercus suber* and holm oak *Quercus rotundifolia* trees in savannah-like grassland, grazed by cattle with some low intensity agriculture. Non-irrigated agriculture, frequently in crop rotation cycles, irrigated agriculture, rice fields, small vineyards and plantations of olive trees and deciduous or evergreen forestry are also present. Apart from the city of Évora (population 56, 600), urban settlements are low in density and in population.

### 3.2.2. Tag deployment and tracking data

White storks were captured using two techniques, a remotely operated, baited clap net and leg lassos constructed of nylon line and rubber and anchored to heavy boards. Both were placed on the landfill and monitored continuously by multiple observers from different locations. Simultaneously with tag deployment, birds were colour ringed on each leg and basic metrics (weight, bill and wing length) were recorded. Birds were retained for a maximum of half an hour. Several individuals were observed in the days immediately after tag deployment and no behavioural of physical effects of the tags were observed. However, as a precaution to allow for tag accustomisation, the first 2 days of data after capture were excluded from this analysis.

Storks were trapped at five landfill sites in the Alentejo, Portugal: Aterro Sanitário Intermunicipal de Évora (38.538004, -7.971274, *n=16* tags deployed), Aterro Sanitário de Vila Ruiva (38.243040, -7.952321, *n=10*), Aterro Sanitário de Ermidas do Sado (38.021444, -8.353319, *n=11*), Aterro Sanitário da Herdade do Montinho, Beja (37.924916, -7.864950, *n=8*) and Aterro Sanitário do Barlavento, Portimão (37.214041, -8.522350,*n=3*). Licenses to catch and tag birds were granted by the Instituto da Conservação da Natureza e das Florestas (ICNF).

New GPS-ACC data loggers, developed by members of our research team, were programmed to collect data 5 times per day at 6am, 9am, 12am, 3pm and 6pm GMT. Each data burst consisted of 8 - 10 consecutive GPS fixes (±20 m accuracy), once per second. Every two days data were transmitted to a web platform via GPRS using the GSM network. Data loggers were deployed back-mounted on a teflon harness. Biodegradable stitching was included to avoid lifelong attachment. Battery powered loggers weighed 90g and solar powered ones weighted 45g, less than 4% of total body weight.

### 3.2.3. Spatial data analyses

Only data bursts from the winter are included in this study, covering the period between the 15th November and 28th February in 2 consecutive winters (2012/13 and 2013/14). The start of the breeding season is defined as the 28th February, which is the earliest first egg of any tagged individual that we recorded.

All data bursts when the bird was in flight are excluded from this analysis. Flight was determined by calculating the mean speed associated with each data burst (minimum 8 seconds of continuous GPS fixes). Speeds ≥2.8km/hr (but typically >20km/hr) were interpreted as flight. The last GPS fix (deemed to be most geospatially accurate) from each non-flight data burst was imported into ArcGIS to calculate the percentage of GPS fixes occurring on non-landfill habitat, landfill or on the nest. GPS fix is here defined as the location most representative of a particular non-flight data burst.

All GPS fixes within a 50m buffer surrounding the perimeter of each landfill were considered as occurring on landfill. Consequently, birds that were temporarily flushed off the active landfill to the site boundary were also included as on landfill.

The roost sites of tagged birds were easily detected as the location of tightly clustered GPS coordinates in continuous use throughout the winter. These locations were visited during the early breeding season to confirm nest occupancy. All birds roosted on their nests and were faithful to the same nest throughout the winter. Birds frequently perch close to, but not on, the nest so all GPS fixes within a 20 m buffer around the nest were considered to be nest attendance. The minimum straight-line distance between the centre of the landfill and the nest was used to calculate the distance between the nest and landfill.

### 3.2.4. Climatic variables

Climatic data from the meteorological station at Évora (latitude: 38.53, longitude: -7.9, altitude: 246 m), accessed from the National Oceanic and Atmospheric Administration (www.noaa.gov) were used in this analysis (Table 3.1). This station is in the centre of the study region and situated on average 47.5 km ±12.4 from the landfill sites. Continuous daily data was available for the entire study period and well represented the climate of the whole area due to the flat topography and homogeneity of the Alentejo region.

Mean climate data for Évora 1981-2010 were obtained from the Instituto Portugues do Mar e da Atmosfera (www.ipma.pt) in order to establish how the study winters compared to long term trends. Rainfall (daily precipitation, moving averages of the previous 10 days and 7 days of precipitation), temperature (mean, maximum and minimum) and mean wind speed were considered likely climatic predictors of winter landfill use by white storks.

The timing and volume of precipitation differed between the two study winters, compared to the 1981-2010 means. In both winters the rains began in late September after anomalously dry Augusts (-80.7 mm and -82.0 mm respectively, anomalous compared to the 1981-2010 mean). Overall, winter 2012/13 was wet, receiving +45.6 mm more precipitation in total compared to the long term average. The majority of rain fell in November (+81.1 mm) and monthly rainfall alternated between being anomalously low and high in successive months.

Winter 2013/14 was overall drier (-20.6mm). The majority of rain fell in October (anomaly: +50.5 mm) and the rest of the winter was very dry (November-February mean anomaly: -64.8 mm), December being especially dry: -88 mm. Periods with more rainfall may coincide with greater prey availability in non-landfill habitats through influencing the height and duration of standing water in flooded meadows. For this reason, the moving average of the precipitation over the previous 10 and 7 days were included in correlation analysis as possible candidates for inclusion in the model. Both winters were cooler than the long term mean (September-February mean, winter 2012/13: -1.1$^{0}$C, winter 2013/14: -0.7$^{0}$C). February being the coldest month in winter 2012/13 with a temperature anomaly of -2.3$^{0}$C. In winter 2013/14 November was coldest with an anomaly of -2.6$^{0}$C.

***Table 3.1*** *All climatic and non-climatic variables considered for inclusion in the model. Climate variables, from the meteorological station at Évora, describe the climate in the study region during the winters of 2012/13 and 2013/14. All climatic variables were measured daily. Mean precipitation is the moving average of precipitation recorded over the previous 10 and 7 days respectively. Mean temperature and mean precipitation 7 were excluded prior to running the model. The identities of landfills 1 to 5 are found in Table 3.2. Nest-landfill distance is the straight line distance between the nest and the landfill site. Winter (1 or 2), landfill ID and individual were included as random effects.*

| | **Mean ±SE** | |
|---|---|---|
| **Variable** | **Winter 1 (2012-13)** | **Winter 2 (2013-14)** |
| **Max Temp $^{0}$C** | 14.6 ± 0.2 | 15.1 ± 0.2 |
| **Mean Temp $^{0}$C** | 9.2 ± 0.8 | 9.6 ± 0.6 |
| **Min Temp $^{0}$C** | 6.1 ± 0.3 | 5.8 ± 0.3 |
| **Daily Precipitation (mm)** | 2.8 ± 0.6 | 2.5 ± 0.5 |
| **Mean Precipitation 10 (mm)** | 2.8 ± 0.3 | 2.5 ± 0.3 |
| **Mean Precipitation 7 (mm)** | 2.8 ± 0.3 | 2.4 ± 0.3 |
| **Mean Wind Speed (m/sec)** | 3.4 ± 0.2 | 3.7 ± 0.2 |
| **Landfill ID** | 1 - 5 | 1 – 5 |
| **Nest-Landfill Distance (km)** | 12.4 ± 2.2 | 15.9 ± 3.4 |
| **Individual (bird identity)** | 1 - 11 | 12 - 25 |

Overall, mean wind speeds were 0.3m/sec faster in winter 2013/14 compared with winter 2012/13. Wind speed was considered an important variable to include because storks are obligate soaring birds and wind speed may influence the development and stability of the thermals on which they rely.

### 3.2.5. Statistical analysis

A binomial Generalized Linear Mixed Model, GLMM, (R package lme4, Bates *et al* 2014) was created-to test the relative influence of climatic and non-climatic variables, and their interactions, on the proportion of daily GPS fixes away from the nest that occurred on landfill during the winter period. The dependent variable was the number of GPS fixes on landfill as a proportion of all foraging GPS fixes constructed using events-trials syntax to account for the fact that there were not always 5 GPS transmissions per day. Thus, the dependent variable combined both the number of events (the daily fixes on landfill) and number of trials (total daily GPS fixes excluding those within 20m of the nest or in flight).

Prior to running the model, a Spearman's rank-order correlation was performed to determine the strength of the relationships between all the independent variables considered for inclusion in the model (Table 3.1). There was a strong, positive linear relationship between mean and minimum temperature (r=0.903, p= <0.001, *n*=1718). Mean temperature was more highly correlated with maximum temperature than was minimum temperature (mean: $r_s$= 0.604, p= <0.001, *n*=1718, min: $r_s$= 0.347, p= <0.001, *n*= 1718), so minimum temperature was selected for inclusion in the models. Similarly, mean precipitation 10 was strongly correlated with mean precipitation 7 ($r_s$= 0.885, p= <0.001, *n*= 1718). Mean precipitation 10 was selected for inclusion in the models because it was slightly less correlated with daily precipitation ($r_s$= 0.488, p= <0.001, *n*= 1718), than was mean precipitation 7 ($r_s$= 0.403, p= <0.001, *n*= 1718). The remaining independent variables (Table 3.1) correlated with each other with a Spearman's rank of <0.7 so were all included in the initial model. All continuous variables were standardized to make the parameter estimates directly comparable. This was done by subtracting the mean, and then dividing by the standard deviation (Schielzeth 2010).

The straight line distance between the nest and the landfill site was included as a non-climatic predictor. Year (2012/13 and 2013/14), landfill identity and individual identity (25 birds) were included as random effects. The names of landfills 1 to 5 and the number of tagged birds visiting each are given in Table 3.2. The landfill variable was included to control for the differing sample sizes of tagged birds visiting each landfill and to control for unquantified differences that may exist between landfills including: number of birds visiting, the volume and frequency of deliveries of organic waste and differences in quality of the surrounding non-landfill habitats, all of which may affect levels of competition for food.

Model simplification and selection was carried out by excluding non-significant explanatory variables. The significance of interaction terms was tested using likelihood ratio tests to compare a null model with the model containing the interaction term. The overall significance of the final model, $R^2_{\mathrm{GLMM(model)}}$, was obtained using R (R Development Core Team 2014). Predicted values were obtained from the binomial GLMM by applying the logit back transformation.

Nest-landfill distance is known to be an important influence on the frequency of landfill use (Figure 2.4), so a second binomial GLMM with a logit link function was created to explore whether nest use is influenced by climatic variables, nest - landfill distance and their interactions. As in the first GLMM, the dependent variable was formulated as an events-trials argument where the number of daily GPS fixes occurring within 20m of the nest (events) were considered as a proportion of all available daily GPS fixes, except those occurring in flight (trials). This model considered all GPS fixes, except those occurring in flight. The independent variables and random effects were as above. All statistical analysis was carried out in R.

### 3.3. Results

A total of 48 tags were deployed, 15 in winter 2012/13 and a further 33 birds were tagged in winter 2013/14. Criteria for inclusion in this study were: i) a minimum of 1 month of data transmission occurring between November and 28th February; ii) a mean ≥3 GPS fixes per day (out of a possible total of 5 daily GPS transmissions) and

iii) sedentary behaviour (faithful to the same roost throughout the winter) rather than itinerant wandering over big areas in Iberia. This was to ensure habitat selection would be meaningfully linked to the climatic predictors derived from Évora meteorological station. As a consequence of criteria iii, the birds in this analysis were most likely adults.

A total of 16 birds had insufficient data prior to 28th February (including one individual who died in a power line collision and another who lost its tag). Three tags did not meet the minimum GPS fixes per day and 4 juvenile birds wandered extensively across Portugal and Spain. Consequently, this study focuses on 25 birds, 11 individuals from winter 2012/13 and 14 birds from winter 2013/14. The mean number of transmission days per bird (± SE) were 73.5 ±7.2 and 65.0 ± 6.3 in 2012/2013 and 2013/2014 respectively.

### 3.3.1. Winter landfill use

A mean of 13.6% ± 1.6 GPS fixes occurred on landfill sites (Figure 3.1). Within this, landfill use by individuals ranged from 0.9 - 29.3% of GPS fixes. A mean (±SE) of 28.2% ± 3.1 GPS fixes occurred within 20 m of the nest and nest use ranged from 5.5 - 63.8% of fixes. The majority of GPS fixes were in non-landfill habitat (58.2% ±2.7) and these occurred within 2 - 4km surrounding the nest (see Chapter 2, Table 2.2).

With the exception of one individual, all birds were faithful to the landfill site closest to the nest and never visited other landfills. This bird predominantly used the closest landfill but visited the second nearest landfill site in 11.6% of GPS fixes that occurred on landfill. Immediately after capture another individual moved to its nest near a new landfill (Avis: -39.092123, -7.729116) and used this landfill exclusively for the rest of the study period. Mean distance travelled between nest and landfill was similar between landfills with the exception of Sado (Table 3.2). Birds visiting this landfill travelled approximately 8.9 km further.

### 3.3.3. Predictors of landfill use

Three predictors of frequency of winter landfill use were retained as significant by the final model (Table 3.3). In order of effect size, the 10 day moving average precipitation was the most significant with a negative effect of 11.5% on probability of landfill attendance. Nest-landfill distance had a negative effect of 10.0% and minimum daily temperature had a negative effect of 5.3%. Predicted values (derived by logit back transformation for a change of one unit (mm, km or $^0$C, as appropriate) were similar and indicated that the probability of landfill use dropped by 2.3% with every 1 mm increase in 10-day running mean precipitation and by 2% with every 1 km of additional distance between the nest and landfill site. Probability of a GPS fix on landfill decreased by 1.1% with every 1$^0$C rise in minimum temperature. No interaction terms were significant.

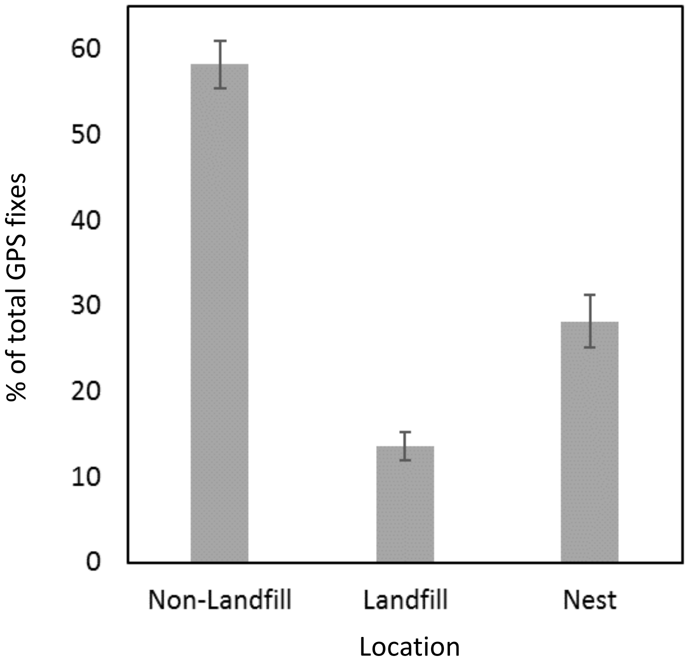

**Figure 3.1** *Percentage of total GPS fixes (±SE, excluding fixes in flight) that occur in non-landfill habitat, on landfill sites and within 20 metres of the nest.*

**Table 3.2** *Landfill sites, the number of birds visiting each landfill and the mean distance between the nest and landfill site.*

| **Number** | **Landfill** | **Number of birds** | **Mean nest – landfill distance (km)** |
|---|---|---|---|
| 1 | Évora | 10 | 11.4 ± 2.6 |
| 2 | Vila Ruiva | 6 | 12.7 ± 2.9 |
| 3 | Avis | 1 | 14.2 ± 0.0 |
| 4 | Beja | 2 | 12.7 ± 1.2 |
| 5 | Sado | 5 | 21.6 ± 7.0 |

**Table 3.3** *Results of the final GLMM explaining the proportion of daily fixes on landfill during winter 2012/13 and 2013/14. Individual, landfill used and year were included as random factors. No interaction terms (italics) were retained in the final model.*

| **Predictors** | **Estimate** | **SE** | ***Z*** | ***Pr(>\|z\|)*** |
|---|---|---|---|---|
| Intercept | -0.962 | 0.040 | -23.938 | <0.001 |
| Mean Precipitation 10 (mm) | -0.115 | 0.025 | -4.644 | <0.001 |
| Nest-landfill distance (km) | -0.100 | 0.041 | -2.421 | 0.010 |
| Minimum temperature ($^{0}$C) | -0.053 | 0.023 | -2.298 | 0.021 |
| | | | | |
| *Interactions (not retained by final model)* | | | | |
| *Ppt 10 – Nest-landfill dist* | *0.045* | *0.025* | *1.823* | *0.063* |
| *Min temp – Nest-landfill dist* | *0.001* | *0.002* | *0.506* | *0.613* |
| *Min temp – Ppt 10* | *0.014* | *0.022* | *0.655* | *0.513* |
| | | | | |
| Random effects | Variance | SD | | |
| Individual | 2.579e-02 | 1.606e-01 | | |
| Landfill Used | 3.290e-08 | 1.814e-04 | | |
| Year | 2.990e-09 | 5.468e-05 | | |

Based on a lack of statistical significance, maximum temperature, daily precipitation and wind speed were removed from the final GLMM used to explain the proportion of daily GPS fixes occurring on landfill sites. Overall, the final model explained 26.3% of the variance ($R^2_{GLMM(model)}$), of which the fixed effect component explained 17.6% of variance.

Storks utilised landfill more frequently during phases of dry weather (10-day running mean precipitation ≤2.12 mm per day, mean ± SE GPS fixes on landfill: 19.1 ± 2.0%) rather than wetter periods (10 day running mean >2.3 mm per day, GPS fixes on landfill: 11.1% ± 1.6, paired t-test: t(24)= -4.933, p= <0.001, Figure 3.2a). Frequency of occurrence on non-landfill habitat was the opposite and increased during wetter phases compared to dry phases (t(24)= 4.727, p= <0.001).

Individuals nesting closer to the landfill site than average (<14.4 km) occurred more often on landfill (mean ± SE 17.2 ± 2.1% versus 10.1% ± 2.8 of GPS fixes on landfill, Wilcoxon signed-rank test: Z= 70.000, p= 0.048) and less often in non-landfill habitat (Z=-2.272, p= 0.023) compared to birds with nests located at higher than average distances from landfill sites (Figure 3.2b). Storks were more likely to use landfill (17.1% ± 2.0 versus 11.9% ± 1.7, Z= -3.771, p= <0.001) and less likely to use non-landfill habitat when temperatures were below average (5.9 $^0$C, Figure 3.2c).

None of the landfill use predictor variables retained by the model influenced the proportion of GPS fixes occurring on the nest during winter (10-day mean precipitation: Sign test, p=0.424, nest-landfill distance: Z= -0.874, p= 0.406, minimum temperature: Z= -0.619, p= 0.536).

### 3.3.4. The influence of climatic variables on winter nest use

The second GLMM investigated if weather variables or nest - landfill distance influence winter nest use. No predictor variables (fixed and random effects, or interactions) were significant in the model output (Appendix 3.1). This indicates that, in winter, resident storks use their nests with equal probability and this is independent of weather or nest – landfill distance.

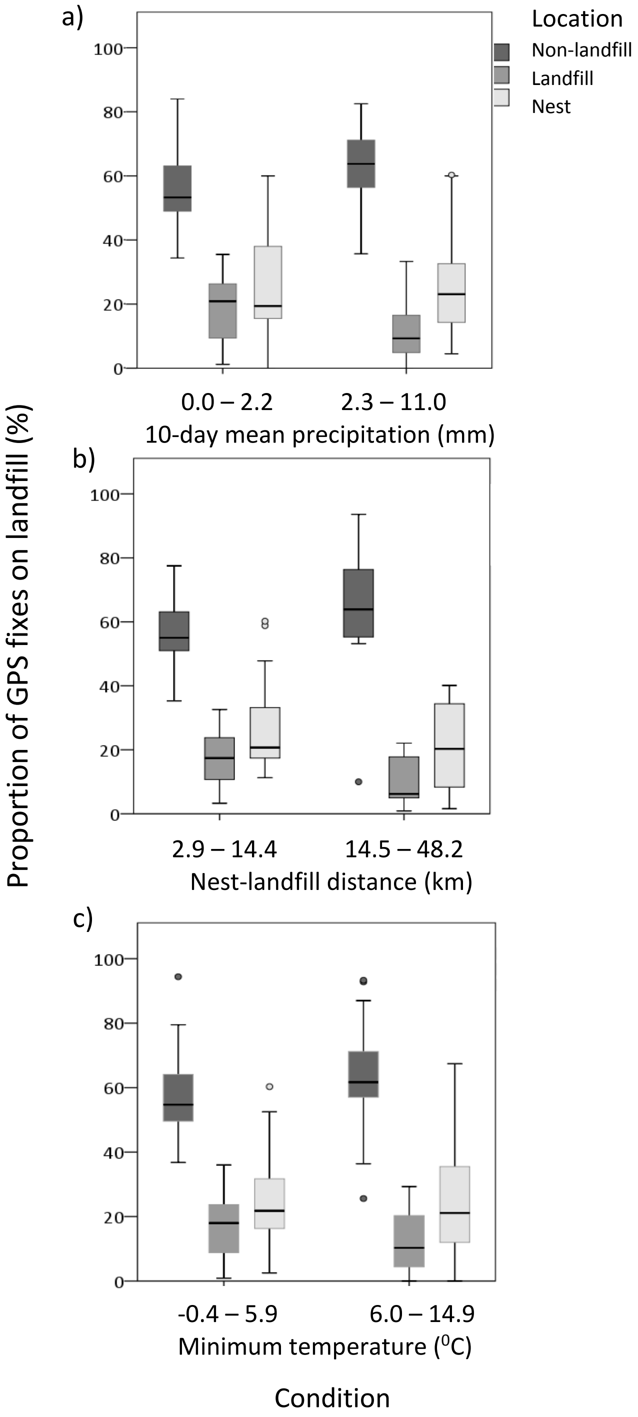

**Figure 3.2** *Comparison of the percentage of GPS fixes occurring in 3 locations (non-landfill, landfill and on the nest) grouped depending on whether conditions in the predictor variables are below or above average. Predictor conditions were: a) distance between nest and landfill, b) daily precipitation (mm) and c) minimum daily temperature ($^{0}$C). The non-landfill habitat category is represented as dark grey, landfill as medium grey and nest as light grey.*

## 3.4. Discussion

### 3.4.1. Climatic predictors of landfill use

Future climatic change in the Mediterranean is anticipated to be severe with high magnitude changes in rainfall and temperature patterns (IPCC 5AR, 2013). In Iberia winter rainfall is predicted to substantially reduce whilst minimum temperatures will increase (Nunes *et al* 2008, Perez and Boscolo 2010). Climate is also predicted to become more variable with an increase in extreme precipitation and heat events (Hoerling *et al* 2012). Although extreme events affect survival, climatic influence on avian populations is primarily indirect through impacts on resources and habitat structure (Newton 1998). The systematic closure of open landfills is already underway in Iberia so it is important to understand which climatic factors are important drivers of landfill use in order to understand how white storks wintering in Iberia will cope with landfill closure and future climatic change.

GLMM results suggest that overall humidity (measured as the 10-day moving average of accumulated precipitation) is the most important factor in predicting the frequency of landfill utilisation. Wetter phases are associated with lower landfill attendance and higher frequency of occurrence on non-landfill habitat (Table 3.3, Figure 3.2a), whilst dry phases are the inverse. Probability of landfill attendance decreases by 2.3% with every additional 1 mm of accumulated 10-day precipitation. Daily precipitation was not significant, therefore was not retained in the final model, indicating that landscape humidity is the important consideration, rather than factors associated with individual rain events, for example reluctance to fly distances during rainfall due to wet feathers or lack of thermals for soaring (Arslangündoğdu *et al* 2011).

It is well known that storks preferentially forage in flooded meadows and irrigated areas (Carrascal *et al* 1993) so it is likely that wet phases promote optimum foraging conditions in non-landfill habitats through influencing the height and duration of standing water in meadows and ditches. Preferred prey species, such as red swamp crayfish and amphibians are also likely to be more active in wetter conditions. During dry phases, prey in non-landfill habitats may be considerably less accessible and require more foraging effort to obtain. For example, the red swamp crayfish, a main prey species, avoids dessication by sheltering in burrows and refugia by day (Ilhéu *et al* 2003). Landfills may currently be buffering white storks against climatic impacts through providing supplemental food sources during dry phases.

Climatic prediction for drier winters with fewer rainy days may therefore be problematic for white storks. In Iberia, lower precipitation will shift rivers from humid to semi-arid catchment regimes. Characteristics will include irregular river flow, increasingly marginal conditions for cultivation (Nunes *et al* 2008) and a reduction in ground vegetation (Gouveia *et al* 2009), which may affect prey abundance. The main alternative food source to landfill, red swamp crayfish, is desiccation tolerant making it suitable for colonisation of ephemeral streams (Cruz and Rebelo 2007). They are already widespread and abundant in all river systems across Iberia, not just in rice fields. However, increased dependence on crayfish will likely favour resident birds (as opposed to migrants) and older, more experienced individuals (Sanz-Aguilar *et al* 2015).

Daily minimum temperature was also a significant predictor of landfill attendance. Storks were more likely to visit landfill rather than non-landfill habitat on days with below average minimum temperature (Table 3.3, Figure 3.2c) and attendance decreased 1.1% per $1^0$C increase in temperature. This may be because prey in non-landfill habitat, such as insects and crayfish, are less active. Additionally, decomposition taking place within the landfill likely makes landfill sites comparatively warmer than the surrounding non-landfill habitat, which reduces exposure to the lowest temperatures. It is known that white storks can tolerate periods of cold (Mata *et al* 2001) but an increasingly mild winter climate will benefit resident birds by reducing energy expenditure in thermoregulation and increasing the activity level of prey species. Iberian minimum winter temperatures are already within the tolerance range of the red swamp crayfish (Huner 2013), but warmer minimum winter temperatures will more suit this prey species, potentially increasing winter survival and therefore overall population numbers. However, significant changes in habitat and agricultural practices are forecast for the Mediterranean in the future due to a more arid climatic regime. For example, climatic change and abandonment of traditional agricultural practices are causing a reduction in the total area of cork oak savannah grassland (montado), another favoured foraging habitat of white storks

(Berrahmouni *et al* 2009, Carnicer *et al* 2011). It remains to be seen what the outcomes of rapid climate and environmental changes will have on the Iberian wintering white stork population.

### 3.4.2. The importance of nest-landfill distance in predicting reliance on landfill

In this study, nest-landfill distance was the second most important predictor of frequency of landfill use, however its effect size was almost equivalent to the accumulated precipitation, suggesting it is equally important. Birds nesting closer to landfill had higher landfill attendance than birds in more distant nests, with probability of a GPS fix occurring on landfill decreasing by 1.1% for every additional 1 km of distance between the nest and landfill.

A surprisingly large proportion of GPS fixes occurred within 20 m of the nest for all birds throughout the winter period (Figure 3.1). Winter use of the nest is rare in temperate climates where resident birds usually undergo local or regional scale movements, often in loose flocks, in search of scarce winter food (Newton 1998). Therefore, remaining in the breeding territory and on the nest during the winter has likely been facilitated by changes in the reliability and abundance of winter food resources, especially from landfill sites.

Breeding season GPS fixes indicated that all tagged birds in this study proceeded to breed in their winter nests *n*= 24 (or in a nest within the same colony, *n*=1). This indicated that our tagged birds were exclusively resident in Iberia, rather than migratory birds from the Eastern European breeding populations, and therefore had a vested interest in maintaining their breeding territory and nest during the winter period. This may explain why frequency of winter nest was not related to climatic factors or distance from landfill (Appendix 3.1).

In order to investigate how white storks are utilising this important food resource, tags were deployed on birds caught exclusively on landfill. The overwintering white stork population is known to concentrate close to landfills (Tortosa *et al* 2002) and the majority, if not all, individuals are believed to feed on landfill to some extent whilst overwintering. Consequently, birds caught on landfill are believed representative of the wintering population. However, it seems likely that the behaviour of resident birds may differ from the behaviour of non-resident overwintering birds.

Resident wintering storks are more likely to be rice field specialists and less reliant on landfill than non-resident birds (Sanz-Aguilar *et al* 2015). This is possibly because, compared to resident birds, migrant individuals are less familiar with the non-landfill habitats in the area and also with techniques specific to local prey species, such as foraging on red swamp crayfish in rice fields. On the other hand, it may be that non-resident birds wintering in Iberia are not under pressure to defend their breeding territory and therefore are able to congregate more exclusively at landfill sites where foraging is optimal (Araújo *et al* 2011). Either way, the GLMM results of this study suggest that for resident, nest holding individuals, the distance between the nest and landfill is currently the significant predictor of frequency landfill use (Table 3.3, Figure 3.2a). Birds nesting closest to landfill use landfill more and will therefore be most impacted by landfill closure. Based on the literature, it is also possible to speculate that non-resident over wintering birds may be more impacted by landfill closure than resident birds. Their lack of familiarity with local habitats will make them more vulnerable to the higher frequency of dry or drought phases predicted by future climate scenarios.

### 3.5. Conclusions

White storks supplement their foraging with landfill during dry periods and on cold days (-0.5 – $6^0$C). This suggests that landfill is increasing winter survival by mitigating the impacts of spells of drought and cold weather. This study predicts that after landfill closure future climatic predictions for warmer, drier winters may make Iberia increasingly unsuitable for overwintering white storks.

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# Chapter 4

## Contrasting long-term productivity trends in new foraging habitats of white stork (*Ciconia ciconia*) in Iberia

To be submitted as:

Gilbert, N.I. Rosa, G., Silva, J.P., Pacheco, C., Correia, R., Catry, I., Gill, J.A. and Franco, A.M.A., Contrasting long-term productivity trends in new foraging habitats of white stork (*Ciconia ciconia*) in Iberia.

## Abstract

In temperate zones foraging resources are unevenly distributed spatially and temporally. Breeding success can vary with habitat quality, because of variation in the quality of resources that those habitats provide. Significant environmental changes associated with anthropogenic activities can also impact foraging opportunities, including the creation of new habitats. Landfill sites provide new foraging resources that are guaranteed in both space and time. White storks *Ciconia ciconia* in Iberia are among the species that have benefited from their utilization both in the winter and in the breeding season, and they increasingly nest close to landfills. However, due to EU directives, open-air landfills must close by 2018 to be replaced by covered recycling facilities. This will likely have consequences for white stork population size and distribution. This study uses data from 48 white storks tagged with high precision GPS/GSM data loggers and 8 years of productivity data from Portugal to investigate 1) which are the most utilized non-landfill foraging habitats and 2) what is the impact on breeding success (percentage of successful nests, and mean number of fledglings per breeding pair) of colony distance from landfill and distance from the most utilized non-landfill habitat.

Compositional analysis of breeding season (March – June) GPS data indicated that rice fields are selected over all other available non-landfill habitats. Other frequently used habitats included non-irrigated and irrigated farm land and cork oak grassland, a traditional Mediterranean agro-forestry system. These habitats will likely be more important for white storks after landfill closure.

Averaged over the 8 study years, nests near landfills had a higher percentage of successful nests and more chicks fledged per breeding attempt than colonies further away. Results of generalized linear models showed the percentage of successful nests decreased by ~7.3% with every additional kilometre away from landfills and the number of chicks per nest (including failed breeding attempts) decreased by ~3.4% per kilometre from landfill. Considering successful nests only, there was also a significant decrease (~2.4%) in the number of fledglings per nest with distance from landfill, indicating that variability in food supply is highly likely to be the explanatory mechanism. This is the first time that the impact of landfill on breeding success has

been measured quantitatively. Our results indicate that landfill closure will have a large impact on breeding success in white storks in Iberia.

There was also variation in productivity in relation to distance from non-landfill habitats. Colonies close to rice fields had higher rates of total nest failure (highest mean ± SE for a single colony over the study period was 76% ± 4.6 of nests failing) in comparison to colonies not close to rice fields. The percentage of successful nests increased by 0.6% per kilometre distance from rice fields. However, the mean number of chicks per nest was similar across habitats indicating that, in successful nests, productivity close to rice fields was comparable to other land use types. This is the first study to assess the implications of rice field habitat on white stork breeding success. As productivity away from landfills was low overall, poor breeding success near rice fields may have important consequences for future white stork population dynamics in Iberia after landfill closure. Possible hypotheses for the differences in nest failure rates in rice fields compared to other habitats are discussed.

## 4.1. Introduction

Habitat quality can affect both the spatial and temporal availability of food resources, and can be a key factor in individual long term fitness and breeding success (Martin 1987, Newton 1998, Sergio *et al* 2009). Regions with resources that are variable in space and time have different species richness and bird biomass (Somveille *et al* 2013) compared to more stable environments. Additionally, foraging strategies will be different in unstable environments where food resources are seasonal, and usually require individuals to be highly mobile (Newton 1998). Landfill sites are a newly available, anthropogenic food resource that is guaranteed year-round, providing stable conditions in an otherwise unstable environment. In Portugal, food waste became available to birds in the 1980s when managed landfill sites were created. Prior to this, domestic waste was dumped and burned locally. The volume of food discarded as waste also increased as at approximately the same time due to economic improvements.

For the white stork *Ciconia ciconia*, food availability for chick provisioning is a main influence on productivity and therefore on population trends (Dallinga and Shoenmakers 1989, Barbraud *et al* 1999, Tryjanowski and Kuzniak 2002), meaning this iconic species is widely regarded as an indicator for habitat quality (Olsson and Rogers 2009, Tobolka *et al* 2012; Janiszewski *et al* 2013).

Food supply has been demonstrated to have a positive influence laying date, clutch size (Tortosa *et al* 2003, Djerdali *et al* 2008, Eggers *et al* 2015) and number of chicks to fledge (Tortosa *et al* 2002, Massemin-Challet *et al* 2006, Kosicki and Indykiewicz 2011) in white storks. Landscapes are a mosaic of habitat patches that vary in quality and white stork productivity has been demonstrated to vary depending on the availability of different habitat types (Tryjanowski *et al* 2005).

Landfill has been utilized by white storks since the 1980s in Iberia (Tortosa *et al* 2002) and more recently, since 1999 in Poland (Kruszyk and Ciach 2006)). It is widely considered to be having a strong impact on population distribution, productivity and survival. Tortosa *et al* (2002), noted that nesting near landfills had a positive influence on the number of chicks to fledge, whilst Massemin-Challet *et al* (2006) found that landfill pairs fledged more chicks than those further (1 km) away from landfills. Storks on the Iberian Peninsula increasingly nest close to landfill sites (Tortosa *et al* 2002), resulting in artificially high concentrations of nests. Chicks fed on food resources from landfill are known to gain weight faster than their counterparts fed a non-landfill diet, which allows them to acquire homeothermy more rapidly (Tortosa *et al* 2002, Tortosa and Castro 2003, Denac 2006). This makes them less vulnerable to severe weather events and reduces mortality (Jovani and Tella 2004).

Landfill colonies are also buffered against the seasonal depletion of food resources and other density-dependent effects observed in non-landfill habitats (Massemin-Challet *et al* 2006). There is also evidence that landfill chicks reproduce at a lower average age (Tortosa *et al* 2002) which speeds up recruitment to the breeding population. Birds that feed on dumps have been known to breed as young as 1 and 2 years of age (compared to the usual age of 3-4 years expected in non-landfill nests (Tortosa *et al* 2002). Assuming lifespan and viable reproductive years are equivalent in landfill and non-landfill populations, landfill individuals potentially have a longer reproductive life and produce more chicks over their lifetime, further enhancing the breeding success of nests associated with landfill.

It is highly likely that feeding on landfill is also facilitating the trend towards year round residency in Iberia, with 80% of overwintering birds located near landfills (Tortosa *et al* 2002) and landfill forming 68.8% of local diets in both adults and juveniles throughout the year (Peris 2003). Consequently, landfill is thought to be an important factor in reversing the severe population decline that continued until the 1970s (Tortosa *et al* 2002, Hilgartner *et al* 2014).

Despite the obvious importance of this new, artificial food resource for the white stork, the reproductive consequences of foraging on landfill have yet to be quantified in detail. This is particularly important because, as a result of EU directives, all open landfills are due to close by 2018. This drastic reduction in food supply could have significant impacts on white stork breeding success, nesting and migratory decisions.

Rice fields represent another new foraging habitat with an abundant prey source that is also extremely important to the white stork and may become increasingly utilized after landfill closure. Rice growing is a relatively recent land use is Iberia. Despite some local scale cultivation occurring in previous centuries, it only became widespread from the 1930s (Lima 1997). Rice growing consists of fields and drainage ditches that are seasonally flooded to a shallow depth, which is ideal for a shallow wading species, such as the white stork, to catch amphibians, insects, fish, reptiles, small birds and mammals. Furthermore, the invasive red swamp crayfish *Procambarus clarkii*, a new prey resource which spread rapidly from Spain in the early 1970s (Tablado *et al* 2010), has been present in vast numbers in both lentic and lotic water bodies, mainly rice paddies and reservoirs, in the study area since the late 1980s (Ilheu *pers com*). White storks are known to feed extensively on crayfish in rice fields during the breeding season and to intensively provision their chicks with them (Negro *et al* 2000, Correia 2001).

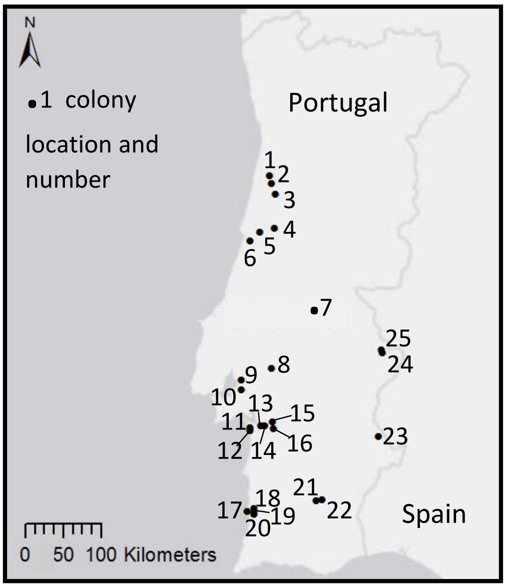
This study investigates variation in productivity as a consequence of relative use of landfill and the most utilized non-landfill habitats in Iberia. The aims are firstly, to establish from individuals tracked with GPS tags which non-landfill habitats are most frequently used; secondly, to compare the impacts of distance between the colony and landfill on productivity (percentage of successful nests and mean number of chicks per nest), and thirdly, to compare productivity in relation to distance to the most used non-landfill habitat.

## 4.2. Methods

### 4.2.1. Study area and species

This study was carried out in colonies located throughout Portugal (Figure 4.1). Rice fields and irrigated agriculture occurs extensively in the local area surrounding colonies in the west of study area, on the estuaries of the Vouga and Mondego rivers to the north and the Tagus and Sado in the south. Other local non-landfill habitat surrounding colonies in the north were mixed, low intensity agriculture in crop rotations with fruit trees and vineyards, wet meadows and large stands of forest. To the south the habitat was mostly a mosaic of low intensity agriculture with irrigated agriculture (including olive groves and vineyards) and extensive areas of cork oak grassland (montado). This traditional, low intensity agro-forestry system consists of cork oak *Quercus suber* and holm oak *Quercus rotundifolia* trees in varying density in savannah grassland with some low intensity agriculture and cattle grazing. Urban areas around the colonies are mostly low density and diffuse.

**Figure 4.1** *Locations of the 25 study colonies in Portugal. Colony numbers correspond to the colony numbers listed in Table 4.1.*



Over the course of this study there was a trend for land abandonment, particularly of the traditional cork oak grassland, and an increase in water bodies and areas of rice paddy, mostly taken from agricultural land (APA 2009).

The climate is classed as temperate continental with warm dry summers (IP 2015) with a gradient of decreasing humidity from west to east and from north to south. South-central Portugal and the Spanish border are particularly hot and dry.

White storks breed asynchronously. In Portugal, white storks usually start laying in the first week of March, laying up to 6 eggs, usually 4 (Zielinski 2002). Eggs hatch 33-34 days later and chicks fledge 58-64 days after hatching. Adults may feed chicks for up to a week after fledging. White storks raise a single brood per year.

### 4.2.2. Tag deployment and assessment of breeding season habitat use

Forty-eight white storks were equipped with GPS-ACC data loggers newly developed by the authors. Tag deployment occurred during the winters of 2012/13 (n=15) and 2013/14 (n=33). Storks were captured on 5 active landfill sites in south-central Portugal (Aterro Sanitário Intermunicipal de Évora (38.538004, -7.971274, *n=16)*, Aterro Sanitário de Ermidas do Sado (38.021444, -8.353319, *n=11*), Aterro Sanitário de Vila Ruiva (38.243040, -7.952321, *n=10*), Aterro Sanitário da Herdade do Montinho, Beja (37.924916, -7.864950, *n=8*) and Aterro Sanitário do Barlavento, Portimão (37.214041, -8.522350, *n=3*)). Storks were captured using leg lassos of nylon and rubber and a remotely activated clap net. Capture and tag deployment was licensed by the Instituto da Conservação da Natureza e das Florestas (ICNF).

Tags collected 5 GPS fixes per day at 6am, 9am, 12am, 3pm and 6pm GMT, each consisting of 10-20 consecutive GPS fixes (±20 m accuracy). Data were transmitted via GPRS using the GSM mobile phone network every 2 days to a web platform. Tags weighed 45g (solar powered) or 90g (battery powered) which was less than 4% of total body mass of each individual. Tags were back-mounted with a teflon harness that contained biodegradable string in order to prevent life-long attachment.

All tagged birds were subsequently resighted on their nests during the breeding season and productivity was monitored using protocols outlined below. White storks do not breed in synchrony so the breeding season was defined as beginning on 1st March (egg laying in early nests) and ending on 30th June (final chicks fledging from late nests). Analysis incorporated habitat use data from tagged birds for the duration of this period. Nests were all located in colonies in south-central and south west Portugal.

Only GPS locations recorded during the breeding season were used in this analysis. GPS fixes occurring during flight were also removed. Flight was recognisable from speed, calculated as the distance between each consecutive GPS generated as part of each transmission. ArcGIS was used to link the GPS points to habitat categories derived from the Corine Land Cover (CLC) 2006 inventory. GPS positions occurring within 20 metres of the nest were considered to be nest attendance rather than foraging so were excluded from habitat analysis. The nest was easily recognised as a continuously used location of tightly grouped GPS coordinates. Since the aim was to establish patterns of habitat use after landfill closure all GPS locations occurring on and within 50 metres of an active landfill site were also excluded. Home ranges of tagged birds in non-landfill habitat were generated from the minimum convex polygon (MCP) of 95% of GPS fixes in order to exclude non-typical behaviour. A compositional analysis (Aebischer *et al* 1993) comparing selected with available habitats was carried out in R using the Adehabitat library (Calenge 2006).

### 4.2.3. Historic trends in fledging success in relation to habitat use

A nation-wide monitoring scheme recorded fledging success over time in selected nests in white stork colonies throughout Portugal between 2005 and 2009. Monitoring was coordinated by the authors, with local assistance from field biologists. Between 2012 – 2014 fledging success was recorded by the authors in 25 of the previously monitored colonies (Figure 4.1, Table 4.1).

A minimum of 2-3 visits were made to each colony during the early and late phases of the breeding season to identify nests occupied by pairs and to capture fledging success in both early and late season nests. Where possible early season (March) visits were made at dusk or dawn to identify pairs roosting on nests and a camera pole was used to check for eggs. Coastal nests could be viewed in directly. It can be difficult to determine if a nest is occupied by a breeding pair so only nests where a pair were present during an early season visit, or eggs were present, are included in order to reduce the risk of over-estimation of nest failures.

Productivity is quantified by three metrics, the percentage of successful nests, the mean number of chicks per nest (including failed breeding attempts) and the mean number of chicks per successful nest. The percentage of successful nests is the number of successful pairs in a colony divided by the total number of monitored pairs. The mean number of chicks per nest is defined as the number of chicks about to fledge from all monitored breeding pairs, including unsuccessful breeding attempts. The mean number of chicks per successful nest considers only nests that fledged at least 1 chick. Chicks fledge at between 54 – 70 days of age (Hanckock *et al* 1992) so only nests with chicks ≥50 days old contributed to the productivity assessment. However, 91% of chick deaths) occur prior to 20 days of age (Jovani and Tella 2004). Nests containing chicks less than 50 days old were assessed in colony visits carried out later in the breeding season. Chicks were counted by telescope and, where possible, by camera pole. In deep nests chicks were not counted until an adult returned to provision the chicks. At this time all chicks usually stand to greet the

**Table 4.1** *Number of nests monitored, distance from colony to landfill and rice field and productivity metrics for the 25 study colonies. Landfill closures caused the colony – landfill distance to change between 2009 and 2012 and affected colonies have the pre 2009 and post 2012 colony – landfill distances listed. Means are the average across all study years (2005-2009, 2012-14).*

| Colony ID | Mean ± SE nests surveyed per year | Landfill - colony distance (km) | Rice field - colony distance (km) | Mean ± SE chicks per nests | Mean ± SE successful nests (%) |
|---|---|---|---|---|---|
| 1 | 31.3 ± 2.8 | 4.6 | 3.3 | 2.6 ± 0.2 | 85.4 ± 2.7 |
| 2 | 21.5 ± 4.8 | 6.7 | 9.1 | 2.3 ± 0.3 | 83.6 ± 6.0 |
| 3 | 26.4 ± 3.7 | 18.0 / 77.1 | 0.5 | 1.7 ± 0.2 | 69.1 ± 8.3 |
| 4 | 69.5 ± 12.5 | 6.0 | 0.0 | 2.4 ± 0.3 | 82.1 ± 3.7 |
| 5 | 21.5 ± 3.4 | 13.9 | 0.0 | 1.3 ± 0.2 | 61.6 ± 9.7 |
| 6 | 28.3 ± 5.4 | 7.7 / 24.6 | 0.0 | 1.4 ± 0.3 | 62.9 ± 10.1 |
| 7 | 26.8 ± 1.4 | 14.4 / 47.9 | 22.5 | 1.7 ± 0.1 | 82.2 ± 2.6 |
| 8 | 45.8 ± 4.3 | 65.1 | 0.2 | 1.5 ± 0.2 | 67.5 ± 5.7 |
| 9 | 49.6 ± 7.8 | 39.0 / 85.3 | 3.8 | 1.1 ± 0.2 | 52.3 ± 8.2 |
| 10 | 21.4 ± 3.9 | 49.3 / 82.7 | 0.0 | 1.0 ± 0.1 | 52.2 ± 4.1 |
| 11 | 19.3 ± 1.1 | 54.8 | 0.1 | 1.0 ± 0.1 | 48.7 ± 6.4 |
| 12 | 13.1 ± 0.7 | 52.1 | 0.1 | 0.6 ± 0.1 | 34.2 ±6.1 |
| 13 | 11.8 ± 0.5 | 49.2 | 0.1 | 1.2 ± 0.2 | 52.3 ± 5.1 |
| 14 | 21.8 ± 1.6 | 47.9 | 0.0 | 0.8 ± 0.1 | 42.6 ± 6.0 |
| 15 | 14.1 ± 2.9 | 48.1 | 0.6 | 1.0 ± 0.2 | 47.7 ± 7.6 |
| 16 | 11.5 ± 1.4 | 40.5 | 0.2 | 0.4 ± 0.1 | 23.8 ± 4.6 |
| 17 | 38.5 ± 4.1 | 49.7 | 33.5 | 1.2 ± 0.1 | 62.0 ± 5.1 |
| 18 | 11.6 ± 3.0 | 48.8 | 26.5 | 1.2 ± 0.2 | 65.7 ± 9.2 |
| 19 | 6.4 ± 1.3 | 46.1 | 30.0 | 1.6 ± 0.2 | 72.7 ± 6.8 |
| 20 | 11.3 ± 2.0 | 44.2 | 36.0 | 1.5 ± 0.2 | 72.8 ± 6.3 |
| 21 | 21.6 ± 3.7 | 27.7 | 35.9 | 1.1 ± 0.2 | 55.7 ± 6.5 |
| 22 | 15.6 ± 0.8 | 25.0 | 41.1 | 1.3 ± 0.1 | 71.6 ± 4.0 |
| 23 | 11.0 ± 0.4 | 45.2 | 54.0 | 1.2 ± 0.1 | 66.4 ± 3.7 |
| 24 | 13.0 ± 2.7 | 43.7 | 21.6 | 1.0 ± 0.2 | 53.0 ± 8.6 |
| 25 | 9.4 ± 1.7 | 42.7 | 24.1 | 1.4 ± 0.2 | 68.5 ± 5.5 |

adult. Only monitored pairs where breeding outcome was known are included in the study.

The study colonies captured variation in locally available foraging habitats and distance to landfill sites and were selected from a range of latitudes to minimise climatic effects associated with location, such as continentality. Additionally, nests were supported by a variety of structures (including trees, purpose built frames, low and high tension pylons, telephone poles, bridge supports and roofs), both within and between colonies, in order to eliminate any effect of supporting structure on breeding success (Hilgartner *et al* 2014). Fortunately, three of these colonies included the nests of tagged birds in 2013, ensuring continuity between the compositional analysis of habitat selection and historic colony productivity.

### 4.2.4. Impact of landfill and non-landfill habitat on productivity

A colony was considered to be landfill influenced if it was 25 km or less from a landfill site. This distance was chosen because data from white storks tagged by the authors (in review, Chapter 2)) indicated that nest defending individuals used landfill less as distance between the nest and landfill increased. The regression describing this relationship during breeding indicated that nests 25 km distant would use landfill in less than 4.7% of total foraging trips (Figure 2.4b). Some tagged individuals at this distance did not use landfill at all during breeding.

Many colonies are located immediately beside rice fields (0 – 0.6 km distance), however, tagged bird data (authors unpublished data) revealed that birds travel further than the immediate habitat around the nest in order to visit both landfill and rice fields. Consequently, a colony was considered to have access to rice field if this resource was present within 4 km of the centre of the colony. This is based on mean distance from the colony to the nearest utilised rice field from tag data.

All distances were defined as the minimum straight line distance between the colony centre and the nearest perimeter of the landfill or closest rice field. A colony is defined as a minimum of 5 monitored nests occurring within a 515m radius of the colony centre. This distance allowed nests on 4 consecutive high tension pylon towers to be considered a colony. Between 2005 – 2009 there was no data on the total number of nests present in any of the monitored colonies.

### 4.3. Statistical analysis

All statistical analyses were carried out in R (R Development Core Team 2014). Linear regressions were used to investigate the relationship between colony scale productivity (percentage of successful nests and mean number of chicks fledged per breeding attempt and per successful nest) and the straight line distance between the colony and either the nearest landfill or nearest rice field. All 25 colonies were included in regressions assessing the impact of distance from landfill, whereas only colonies located at least 25 km from landfill sites were included in the regressions investigating the effect of distance from rice field. Landfills were excluded to explore productivity in non-landfill habitats in order to understand the possible impact of landfill closures on breeding success.

Three models were constructed to test the effect of distance from landfill and rice fields (and their interaction) on productivity in the 25 study colonies. In one model the relationship between percentage of successful nests in the colony and distance from landfill and/or rice fields was explored using a binomial generalized linear mixed model (R package lme4, Bates *et al* 2014). Percentage of successful nests, was modelled with a binomial “success” or “fail” dependent variable for each nest. Success denoted that at least once chick fledged the nest (ie at least one chick of ≥50 days old was present in the nest), whereas fail indicated that the nest of a breeding pair fledged no chicks. The independent variables were: distance from colony to nearest landfill distance, distance from colony to nearest rice fields and the interaction between the two distance variables for 25 colonies (for details see Table 4.2). Year was included as a random factor. Both distance variables were standardized by subtracting the mean, then dividing by the standard deviation (Schielzeth 2010).

The next two models investigated the impact of distance from landfill and/or rice fields on productivity at nest scale. Firstly, the number of chicks fledged per nest (including breeding attempts that failed) was investigated using a Poisson regression model. Secondly, the impact of nest distance from landfill and/or rice fields on the number of chicks per successful nest (excluding breeding attempts that failed) was explored using a Gaussian model. In both nest-scale models, year and colony identity (Table 4.2) were included as random factors.

All independent variables were initially included in the models and the statistical significance of each (and their interaction) was tested by comparison to a null model (from which the variable of interest had been deleted) using likelihood ratio tests. A logit link back transformation (binomial model) and a log link transformation (Poisson model) were applied to the final model outputs to derive predicted values. The overall significance of the final models, $R^2_{GLMM(model)}$, was obtained using R (R Development Core Team 2014).

## 4.4. Results

The storks with tags that stopped transmitting before the breeding season (n=20) and others (n=8) suspected juveniles or non-nest holding birds were not included in the analyses. In total GPS data from 20 nest-defending tagged birds (mean transmissions per bird = 406.8 ± 30.2) were used to construct the breeding season compositional analysis ranking matrix (Table 4.1).

**Table 4.2** *caption. Compositional analysis comparing proportions of utilized habitat with proportions of total habitat types available within the home range MCP. The habitat rank indicates whether the habitat type in the row is used significantly more / less than the habitat type in the column. Triple symbols indicate statistically significant preferences.*

**Table 4.2**. *Compositional analysis ranking matrix. Caption bottom of previous page.*

| **Rank**<br>(Score) | **Habitat** | **Rice field** | **Non Irrigated arable** | **Permanently Irrigated** | **Agroforestry** | **Water margins** | **Vineyards** | **Permanent and annual crop** | **Complex cultivation** | **Agriculture & grassland** | **Transitional woodland** | **Unsuitable** | **Olive groves** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **1**<br>(11) | **Rice field** |  | + | + | +++ | +++ | +++ | +++ | +++ | + | +++ | +++ | +++ |
| **2**<br>(10) | **Non Irrigated Arable** | - |  | + | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ |
| **3**<br>(9) | **Permanently Irrigated** | - | - |  | + | + | + | + | + | + | +++ | + | +++ |
| **4**<br>(8) | **Cork Oak grassland** | --- | --- | - |  | + | + | + | + | +++ | +++ | +++ | +++ |
| **5**<br>(7) | **Water margins** | --- | --- | - | - |  | +++ | +++ | + | + | + | + | +++ |
| **6**<br>(5) | **Vineyards** | --- | --- | - | - | --- |  | + | - | + | + | + | + |
| **7**<br>(4) | **Permanent & annual crop** | --- | --- | - | - | --- | - | - | + | + | + | - | + |
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| **7**<br>(4) | **Complex Cultivation** | --- | --- | - | - | - | + | - |  | - | + | + | + |
| **9**<br>(3) | **Agriculture & Grassland** | - | --- | - | --- | - | - | - | + |  | - | + | + |
| **9**<br>(3) | **Transitional Woodland** | --- | --- | --- | --- | - | - | - | - | + |  | + | + |
| **11**<br>(2) | **Unsuitable** | --- | --- | - | --- | - | - | + | - | - | - |  | + |
| **12**<br>(0) | **Olive groves** | --- | --- | - | --- | --- | - | + | - | - | - | - |  |

### 4.4.1. Non-landfill habitat preference

CLC 2006 habitat classifications within the non-landfill MCPs of each individual were summarised into a total of 12 habitat types (Table 4.2). Compositional analysis indicated that, in MCPs where rice field was available, it was positively selected over all other habitat types (Table 4.2). In order of utilization preference, habitats were ranked rice field > non-irrigated arable > permanently irrigated land > cork oak grassland > water margins. There was then a drop in rank score to the remaining 7 habitats, indicating these habitat types were less used. Rice field occurred in the MCPs of 9 birds, suggesting good representation in the analysis. Cork oak grassland and non-irrigated arable land were the most common land use categories, occurring in the home ranges of all birds. In one case, non-irrigated arable was the only available habitat category in the non-landfill foraging range. Habitat types in the unsuitable category were urban and industrial areas and coniferous forest.

### 4.4.2. Productivity trends

#### 4.4.2.1. Historic productivity

Over the 8 study years, breeding success data were collected from 4285 breeding events in 25 colonies. The number of nests monitored per year ranged from 265 in 2012 to 371 in 2009 (mean ±SE number of nests per year = 307.3 ± 12.8). Details of the mean number of nests per colony can be found in Table 4.1. The number of fledglings per nest ranged from 0 to 6 chicks.

Colonies located up to 25 kilometres in a straight line distance away from an active landfill were considered to be landfill-influenced (n =7 colonies, mean ± SE = 147.1 ±10.6 nests). Colonies within 4 km of rice fields were considered to be rice field colonies (n =14 colonies, 230.3 ± 16.7 nests), 5 of which were close to both rice fields and landfill. The remaining colonies (n =9 colonies, 147.5± 5.8 nests) were considered to be out of foraging range of both landfill (mean distance ±SE= 41.4 km± 3.0) and rice field (mean = 33.6 km ±3.3). These colonies were situated in areas dominated by cork oak grassland with some non-irrigated agriculture. Distances from both landfill and rice fields for each colony are found in Table 4.1.

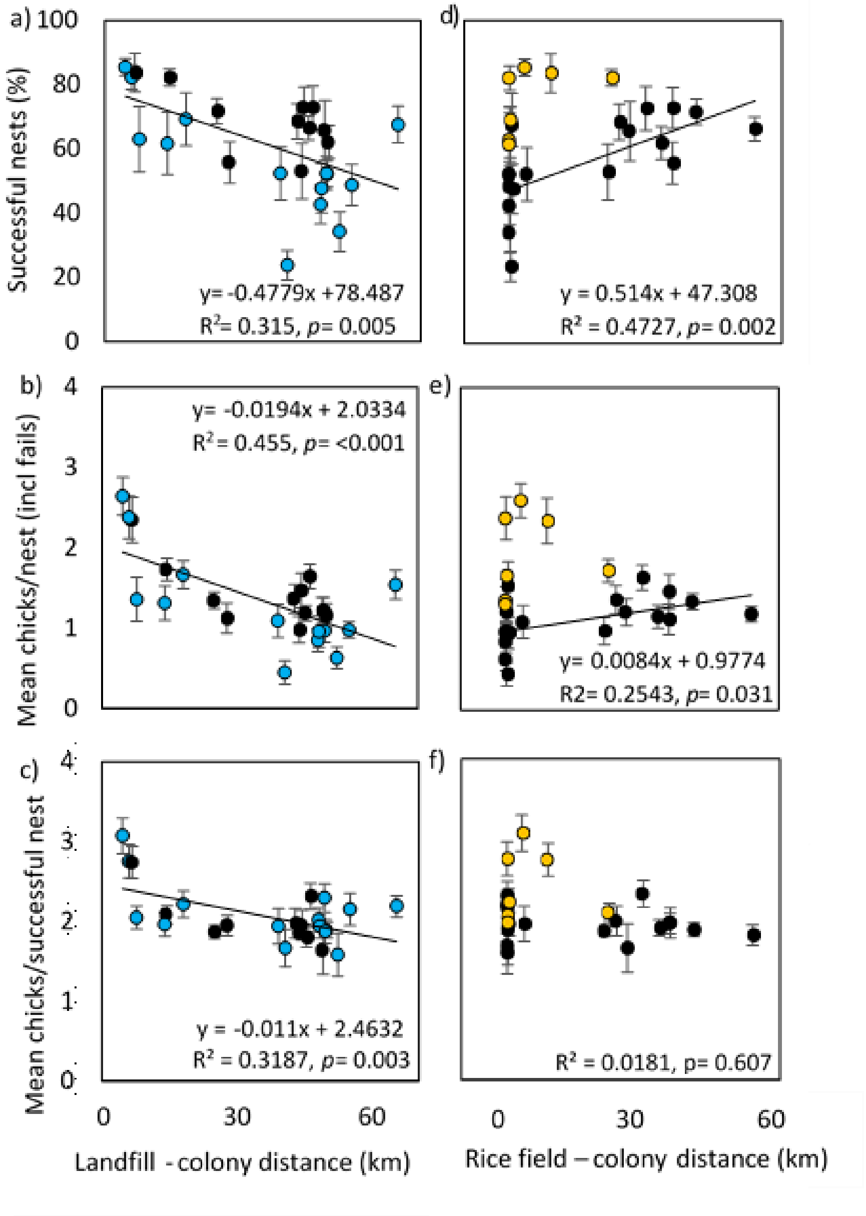


**Figure 4.2** *Mean ± SE colony-scale productivity in relation to the distance between the colony and the nearest active landfill (a - c) and distance between colony and nearest rice fields (d -f), averaged over the years 2005 – 2009 and 2012 – 2014 for 25 colonies. Percentage of successful nests (a, d) expresses the percentage of breeding pairs in each colony that successfully fledged at least one chick. The mean number of fledglings per nest per colony is presented inclusive of failed breeding attempts (graphs b and e) and mean chicks fledged from successful nests only (fledging a minimum of 1 chick, c and f). Colonies identified as blue symbols were located within 4 km of rice fields, colonies in yellow were located within 25 km of landfill. All colonies are included in the regressions in a-c, whereas landfill colonies are excluded from regressions in d-f to assess productivity away from the influence of landfill.*

#### 4.4.2.2. Productivity trends in different foraging habitats

Averaged across all study years, there was a strong, negative relationship between distance from colony to landfill site and i) the percentage of successful nests per colony ii) the mean number of fledglings per nest (including unsuccessful nests) and iii) the mean number of fledglings per successful nest (excluding failed nests), Figure 4.2 a, b and c (see Appendix 4.1 a and b for data presented per year). Colonies situated close to landfill were more likely to successfully rear at least one chick and fledge higher numbers of chicks per nest (both including and excluding failed nests) than colonies where individuals were far from landfill. As a result, over the study period, the mean number of fledglings per breeding pair (± SE) in colonies located within 25 km of landfill (n=7, mean distance 10.2 km ± 2.0) was 2.0 ± 0.2 compared to 1.1 ± 0.1 in colonies located far from landfills (n=18, mean distance 45.5 km ± 2.1). This difference includes the failed breeding attempts.

With the exception of colonies influenced by landfill (in yellow, excluded from regressions in Figure 4.2 d, e and f), colonies located near rice fields experienced highly variable breeding success. Some colonies had high rates of nest failure, Figure 4.2 d (lowest success rate was 34.2% ± 6.1 of nests in a single colony, averaged over the study period, Table 4.1) and low numbers of fledglings per nest (Figures 4.2 e), whilst others performed equivalently to colonies in other non-landfill habitats. As a result, away from rice fields there was a significant increase in both the percentage of successful nests and the mean number of chicks per nest. Taking into account failed breeding attempts, colonies located within 4km of rice fields (mean distance 0.6 km ± 0.4) and far from landfills fledged a mean of 1.0 ± 0.1 chicks per nest, whereas nests far from both rice (33.6 km ± 3.3) and landfill fledged 1.3 ± 0.1 chicks per nest. Landfill colonies excluded, there was no significant effect of proximity to rice field on the mean number of chicks per successful nest (Figure 4.2 f). This means productivity in successful nests is similar across all non-landfill habitats.

Back-transformation of the standardized model results shown in Table 4.3 revealed that, across all 25 colonies, the percentage of successful nests decreased by 7.3% per kilometre away from landfill sites, whilst in colonies near rice fields the percentage of successful nests was 2.6% lower than in other habitats (Table 4.3a). The interaction term between rice field-colony distance and landfill-colony distance

**Table 4.3** *Results of GLMMs predicting the relationship between a) percentage of successful nests per colony, b) mean number of fledglings per nest (including failed breeding attempts) and c) mean number of fledglings per nest (excluding failed breeding attempts) in relation to distance to nearest landfill and/or rice field for 25 colonies. Non-significant effects (not included in the final model) are in italics.*

### a) Successful nests (%)

| **Predictors** | **Estimate** | **SE** | ***Z*** | ***Pr(>\|z\|)*** |
|---|---|---|---|---|
| Intercept | 0.570 | 0.135 | 4.231 | <0.001 |
| Landfill – colony distance (km) | -0.326 | 0.028 | -11.526 | <0.001 |
| Rice – colony distance (km) | 0.075 | 0.036 | 2.121 | 0.034 |
| *Landfill – rice interaction* | *0.039* | *0.005* | *0.694* | *0.488* |
| Random effect | Variance | SD | | |
| Year | 0.136 | 0.369 | | |

Variance explained ($R^2_{GLMM(model)}$): 65.3%

### b) Number of fledglings per breeding pair (including failed nests)

| **Predictors** | **Estimate** | **SE** | ***Z*** | ***Pr(>\|z\|)*** |
|---|---|---|---|---|
| Intercept | 0.196 | 0.115 | 1.687 | 0.091 |
| Landfill – colony distance (km) | -0.249 | 0.075 | -3.388 | <0.001 |
| *Rice – colony distance (km)* | *-0.038* | *0.061* | *-0.618* | *0.537* |
| *Landfill – rice interaction* | *0.050* | *0.093* | *0.538* | *0.590* |
| Random effect | Variance | SD | | |
| Year | 0.762 | 0.276 | | |
| Colony | 0.081 | 0.284 | | |

Variance explained ($R^2_{GLMM(model)}$): 81.6%

**c) Number of fledglings per successful nest**

| **Predictors** | **Estimate** | **SE** | ***Z*** | ***Pr(>\|z\|)*** |
|---|---|---|---|---|
| Intercept | -2.15 | 0.115 | 18.723 | 0.075 |
| Landfill – colony distance (km) | -0.220 | 0.065 | -3.366 | <0.001 |
| *Rice – colony distance (km)* | *-0.115* | *0.060* | *-1.915* | *0.064* |
| *Landfill – rice interaction* | *0.066* | *0.082* | *0.766* | *0.415* |
| Random effect | Variance | SD | | |
| Year | 0.576 | 0.282 | | |
| Colony | 0.576 | 0.240 | | |

Variance explained ($R^2_{GLMM(model)}$): 74.5%

was not significant indicating that rice field habitat use away from landfill does not significantly impact the percentage of successful breeding attempts. In all models, the random effects were not significant, indicating that there was no effect of year or colony identity.

The mean number of fledglings per nest decreased by 3.4% per kilometre distance from landfill (failed nests included) or 2.4% per kilometre (successful nests only), highlighting that the benefit of nesting near landfill is not only increased likelihood of breeding successfully, but also increased likelihood of raising more chicks. The impact of proximity to rice fields on mean number of chicks per nest was only significant when considering failed nests (Table 4.3 e). There was no significant difference in the mean number of chicks per successful nest (Table 4.3 f). Thus, despite higher rates of total nest failure, the number of fledglings raised in successful nests by rice field colonies was not significantly different from the number of chicks raised in other habitats. This was explored further by comparing the frequency and distribution of chicks in nests nest dimensions (ie frequency of occurrence of nests with 0, 1, 2 etc chicks per colony) in different habitats (Figure 4.3).

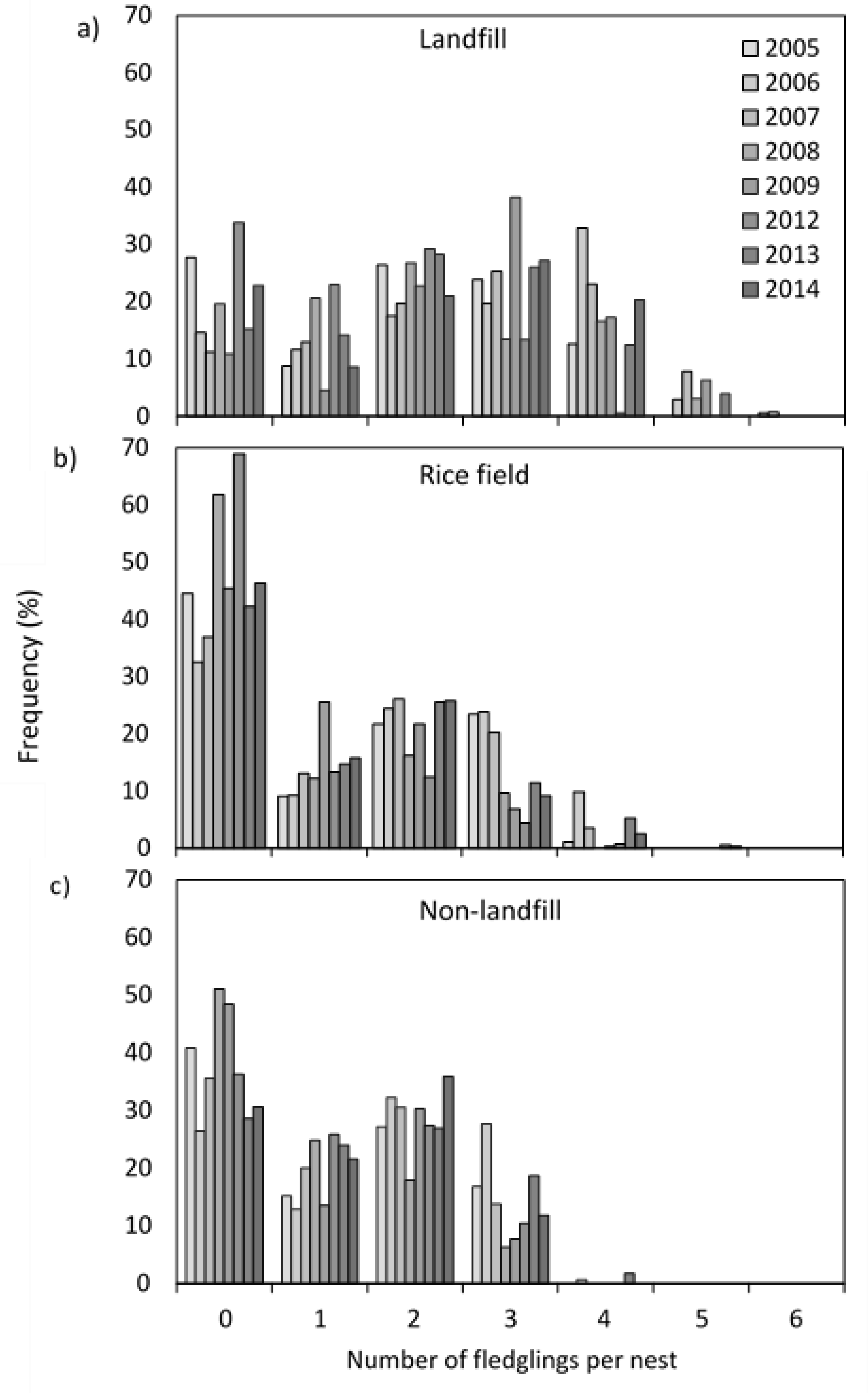


**Figure 4.3** *Frequency distribution of numbers of chicks per breeding attempt in 8 years (2005 – 2009, and 2012 – 2014) for a) colonies close to landfill (colony – landfill distance <8 km, n=4), b) colonies close to rice fields (colony – rice field distance <0.6 km, n= 11) and c) colonies (n=10) far from both landfill (minimum distance = 14.4 km, mean ±SE= 38.7 km± 3.8) and rice fields (minimum= 21.6 km, mean = 32.5 km ±3.1).*

### 4.4.3. Variation in numbers of chicks per nest

The benefit of landfill manifests not only in terms of fewer nest experiencing total failure (no fledglings), but also in the considerably higher frequency of nests fledging 4 or more chicks in comparison to colonies in non-landfill habitats (Figure 4.3). The only nests to fledge 6 chicks were in landfill colonies. This combination of high success rate and high numbers of chicks per nest means that landfill closures will have a considerable impact on white stork productivity.

In congruence with the model results, the frequency of breeding pairs failing to produce fledglings is highest in colonies close to rice fields and this is consistent across study years (Figure 4.3b). The frequency of nests recording 1, 2, or 3 chicks is similar between rice fields and other non-landfill habitats, whereas the frequency of 4 chicks per nest is higher in rice fields (Figure 4.3 b and c). This indicates that whilst many pairs in rice colonies fail completely, a small number of pairs are highly successful.

## 4.5. Discussion

### 4.5.1. Preferred non-landfill foraging habitats

To our knowledge, this study is the first high-precision analysis of white stork habitat selection from tagging data, which has enabled us to rank habitats with very different foraging opportunities in order of utilization preference. This is relevant to our understanding of how the artificially high concentration of storks nesting in the vicinity of landfills may redistribute themselves after landfill closures. The top ranked habitats are congruent with previous studies of white stork habitat selection in Iberia. These indicated dry and flooded pastures, including cork oak grassland, (Alonso *et al* 1991, Carrascal *et al* 1993) and rice fields (Sans-Aguilar *et al* 2014) as key foraging habitats, whilst woods and scrub are avoided.

Food availability is a key driver in white stork habitat selection (Alonso *et al* 1991). White storks are highly opportunistic and, according to theories of optimal foraging, individuals should forage on the most profitable resources (Sutherland 2002). Although it is not known how close to optimal is the white stork foraging strategy, they are known, to some extent, to discriminate between high and low quality patches and those with low and high associated flight costs (Johst *et al* 2011). Rice field habitat is clearly highly important for foraging white storks because compositional analysis indicated that it is selected over all other available habitats (Table 4.2).

Data from tagged white storks (authors unpublished data) indicated that individuals may travel further to visit rice fields than the normal range of breeding season foraging trips. The majority of foraging normally takes place in the immediate 1 - 2.5 km radius around the nest (Dziewiaty 1992, Moritzi *et al* 2001, Denac 2006, Vrezec 2009), but one tagged bird regularly travelled 8 km to foraging in rice fields, whilst another occasionally travelled 13 km. This distance is equivalent to distances others have reported that birds travelled to visit landfill (Tortosa *et al* 2002). The abundance and large size of individual crayfish likely equates to shorter foraging bouts to meet calorific needs.  This suggest that, due to the predictability and abundance of crayfish, after landfill closure white storks may preferentially elect to nest close to paddy fields particularly, but also irrigated areas and water margins. Large flocks of storks on rice and irrigated fields are problematic because they trample seedling crops and muddy the water, which may lead to conflict with local farmers.

The use of arable land by white storks varies throughout its breeding range. Intensification of farming practices have been highlighted as a causal factor in the decline and loss of the white stork across its breeding range (Olsson and Rogers 2009, Janiszewski *et al 2014*). However, in less intensively farmed areas, studies report that storks use arable fields (eg Ozgo and Bogucki 1996, Latus and Kujawa 2005) and that they can be seasonally optimal, such as during ploughing (Pinowska and Pinowski 1989). Farming practices in the study area, Portugal, are generally much less intensive than elsewhere, and often include 5 – 7 year crop rotation cycles with fallow years and grazing. For this reason, the non-irrigated arable category is extremely broad, both temporally and spatially, and suitable for storks because it includes seasonally wet land and grazing, as well as low intensity crops with high abundance of insects, mammals and amphibians.

### 4.5.2. Productivity trends in relation to landfill and rice fields

Iberia is an area of high white stork population density (Thomsen 2006) and results presented here show the impact of proximity to landfill on breeding success to be considerable. Colonies situated near landfills had higher percentages of successful nests and fledged higher numbers of chicks per nest (including much higher frequency of broods of 4 or more chicks) compared with colonies far from landfills (Figure 4.2, Table 4.3, Figure 4.3). The percentage of successful nests decreased by 7.3% per additional kilometre distance from landfill and the number of fledglings per nest decreased by 3.4% per additional kilometre (including failed nests) or 2.4% considering successful nests only. This last metric considers factors impacting breeding success after hatching (therefore eliminates differences in fecundity, and other confounding effects on hatching success such as predation and weather effects). Nest close to landfill have significantly higher numbers of fledglings per successful nest than nests further away (Figure 4.2 c) which indicates that food availability is likely an explanatory mechanism.

To our knowledge, no other study has quantitatively estimated the impact of landfill on productivity, but studies investigating the impact of supplemental feeding in white storks have been carried out. Contrary to our findings, Moritzi *et al* (2001) found no effect of supplemental feeding, which may have been related to favourable foraging conditions in local habitats due to good weather. Hilgartner *et al* (2014), the only study thus far to quantify supplemental feeding effects, reported a drop in number of fledglings per nest of 8% per additional kilometre distance from the feeding station. However, these works are not directly comparable with the current study because feeding stations were only available during the course of the study and only stocked at certain times meaning additional food was not available *ad libitum* as it is in landfills. Furthermore, they were carried out in an area of considerably lower population, meaning population density effects were likely less important.

It is likely that all white storks in Iberia, including those nesting far from landfill, utilise landfill to some extent, particularly during winter. Even occasional landfill use may have carryover effects that improve productivity in the subsequent breeding season, so our estimates for productivity after landfill closure, based on current productivity in colonies far from landfills, may be overestimated.

Nevertheless, our study indicates that landfill closure will have huge consequences for future white stork breeding success because it is likely the high productivity in landfill colonies that is driving the substantial population increase seen in recent decades. Away from landfills (mean 45.5 km ± 2.1), colonies fledged fewer chicks per nest than reported elsewhere in Europe (2005-9, 2012-14 mean ± SE = 1.1 ± 0.1). For example, in a non-landfill population in western France, the mean number of fledglings per nest was 3.2 ± 1.1 (years 1978 – 96, Barbraud *et al* 1999). However, the annual mean fell over the study period in association with density dependent effects as the population increased. By the end of the study the mean was 2.1 ± 1.1, still higher than reported for Portugal by this study. A study in Poland (1983 -2002), reported mean fledglings per breeding pair of 2.58 ± 0.10 and 2.76 ± 0.09 (Tryjanowski *et al* 2004), whilst in Germany 1.7 fledglings per breeding pair was reported (Hilgartner *et al* 2014).

Overall, breeding outputs in the western European population of white storks are low and not dissimilar to the reproductive rates reported during the strong population decline of the 1970s (Hilgartner *et al* 2014). Comparatively low rates of reproductive success in nests away from landfills reported by this study mean it is unknown if non-landfill productivity is sufficient to sustain a stable population in Iberia, particularly in the face of environmental and climatic change in both Iberia (Giorgi 2006, Perez and Boscolo 2010, Hoerling *et al* 2012) and the African wintering grounds (Kanyamibwa *et al* 1990, Saether *et al* 2006, Wilson and Cresswell 2006). Low breeding success is likely due in part to the strong effect of high population density on productivity. In non-landfill colonies, whilst there was no variation in the number of chicks fledged from successful nests (Figure 4.2 f), there was high variation in rates of total nest failure (Figure 4.2 d), and effect that may become increasingly important after landfill closures.

Across all study years, colonies within 4 km of rice field had highly variable breeding success, with some colonies significantly more likely to experience complete nest failure than colonies in other habitats (Figure 4.2, Table 4.3, Figure 4.3). This was surprising because rice fields were the highest ranked foraging habitat (Table 4.2) with an abundant prey source, the red swamp crayfish, that is considered to be of high nutritional value (Negro *et al* 2000). This should make rice fields high quality nesting territories with higher than average breeding success and fewer nest failures. Other studies have demonstrated that nests near wetlands have lower incidence of brood reduction and higher breeding success than those in drier habitats (Barbraud *et al* 1999, Janiszewski *et al* 2013). As rice fields are likely to be utilized increasingly after landfill closure, it is important to understand the causal mechanisms behind the highly variable breeding success in this habitat.

### 4.5.3. Hypotheses for variable productivity in colonies near rice fields

Poor breeding success can result from two occurrences, either low initial clutch size (low female quality or resource availability), or mortality during chick rearing. Insufficient nests were easily accessible and colonies were not visited with sufficient regularity to confirm at which stage the majority of losses occurred. However, views into accessible nests in rice field colonies, and the occurrence of eggshell below, indicated that pairs in rice field colonies were hatching clutches within expected size dimensions (2-5 eggs, authors unpublished data). This suggested that nest failures were likely occurring mainly during the chick rearing phase.

Nest predation in white storks in Iberia is extremely low at under 4% (Vergara *et al* 2006, Aguirre and Vergara 2009, authors' observations) and the colonies near rice field did not differ from other colonies located elsewhere, thus this was considered an unlikely explanatory mechanism. Climatic variables, particularly precipitation and temperature, are known to influence white stork breeding success (Denac *et al* 2006) but total nest failures in rice field colonies were consistently high across all eight study years (Figure 4.3, Appendix 4.1 a and b), encompassing good and bad breeding years. Additionally, colonies at equivalent latitudes and distances from the coast that were not close to rice fields were not impacted suggesting a location specific driver. Prey availability is an important mechanism governing breeding success (Tryjanowski and Kuzniak 2002), therefore, four non-exclusive hypotheses linked to food availability were considered to explain the high nest failure rate close to landfills.

Firstly, inadequate skills in rice field foraging, particularly in dealing with crayfish. Although storks are known to consume crayfish in large quantities (Tablado *et al* 2010), crayfish is a relatively new prey that has colonised the study area within the life time of individuals (~25 years) and is not available outside of Iberia. There is evidence to suggest that expertise and specialism in crayfish develops with age in storks (Sanz-Aguilar *et al* 2015) so young birds may be less adept at foraging in this habitat and therefore chicks suffer lower provisioning rates and higher incidence of starvation or brood size adjustment by the adults (Lack 1954, Mock and Forbes 1994). However, chicks were noted to have orange skin, indicative of carotenoids derived from high crayfish consumption (Negro *et al* 2000). Furthermore, the availability of vertebrates, such as frogs, in wetland habitats means these environments are calculated to have a higher biomass intake than drier areas (Pinowska and Pinowski 1989).

Secondly, some rice field colonies may contain many high proportions of low quality individuals, or young birds breeding for the first time. Juveniles are known to occupy lower quality nest sites in marginal locations and to have lower rates of breeding success and higher risk of total nest failure (Vergara *et al* 2006, Nevoux *et al* 2008). This may result in differential representation of first time breeders in some colonies. In our population there were insufficient birds of known age to draw conclusions about the distribution of age classes within a colony. Furthermore, some low performing rice field colonies were located in areas of perceived good quality foraging habitat, including extensive areas of cork oak and grazing, making it unlikely that every colony was in marginal habitat. However, other low productivity colonies had significant stands of coniferous forest in the immediate vicinity, making these more likely to be marginal zones. Colonies where rice field constitutes the only suitable foraging habitat will be vulnerable to changes in the timings of rice field management (eg flooding, ploughing) because crayfish accessibility is dependent on water level (Cook *et al* 2014). This is because crayfish hide in burrows and other refugia by day to prevent desiccation, whereas storks are primarily day time foragers.

Thirdly, breeding success in certain rice field colonies may be impacted by pollutants, for example heavy metals. Rice fields are located on estuaries down stream of industrial areas that were historically unregulated. This may have led to the sedimentation and accumulation of environmental contaminants in rice fields. Wading birds can ingest large quantities of sediment during foraging and some contaminants, for example lead, can be toxic even in small quantities (Pain *et al*

1998). Heavy metals are known to bio accumulate in apex predators, such as white storks (Bosveld *et al* 1995), with several ciconiform species suspected to demonstrate increased sensitivity (Goutner *et al* 2011). They are also known to accumulate in the main prey species, the red swamp crayfish (Alikhan *et al* 1990, Geiger *et al* 2005, Alcorlo *et al* 2006). Accumulation may influence individual condition with consequences for survival and reproductive success (Heath and Frederick 2005).

Structural deformities associated with heavy contamination were not noted in the study region, however, metal burdens can impact on DNA, hatching success and the development of chicks (Pastor *et al* 2001, De Luca-Abbott *et al* 2001, Boas *et al* 2006). Additionally, the synergistic and antagonistic effect of multiple heavy metals is known to be greater than their individual toxicity (Pain *et al* 1998, Boas *et al* 2006). The combined impact of metal burdens and environmental stressors (eg climatic conditions, poor food availability) on body condition is also known to be important (De Luca-Abbott et al 2001, Boas *et al* 2006), so it is possible that low level contamination may be contributing to making rice field colonies more prone to failure. Future work should investigate this hypothesis further.

### 4.6. Conclusions

Two new, abundant foraging opportunities have become available to white storks within the lifetimes of individuals: foraging on landfill and on the invasive red swamp crayfish in rice fields. Currently in Iberia white stork preferentially nest near landfill sites where productivity is high and this is contributing significantly to the population increase seen in Iberia in recent decades. It is highly likely that rice fields will become key areas for white storks after landfill closure, however, productivity trends in this habitat were highly variable in comparison to other non-landfill habitats, with many nests failing completely. Away from landfill, mean productivity in the study area was relatively low, compared to other European countries, likely the result of both behavioural and environmental factors. It is not known if current levels of reproductive success in non-landfill habitats are sufficient to sustain a viable population. It is thus important to unravel the mechanisms behind differences in breeding success in non-landfill habitats since this information will be essential to understand population dynamics after landfill closure.

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# Chapter 5

## The impact of habitat selection on heavy metals from eggshells in White Storks (*Ciconia ciconia)* in Iberia

To be submitted as a short communication as:

Gilbert, N.I., Silva, J.P., Correia, R.A., Pacheco, C., Catry, I., and Franco, A.M.A., The impact of habitat selection on heavy metals from eggshells in White Storks (*Ciconia ciconia)* in Iberia.

## Abstract

Apex predators are susceptible to the accumulation of pollutants, mainly through consumption of contaminated food sources which biomagnify with progress up the food chain. This can have a detrimental effect on breeding success through impacts on egg and chick development. On the Iberian Peninsula, the population of white storks *Ciconia ciconia* is known to forage extensively on landfill sites and rice fields throughout the year. Both these sites may be contaminated due to environmental pollution. Landfill food resources may be in contact with toxic rubbish and rice fields may have trace elements deposited in sediments. In Portugal, breeding success in colonies located near some rice fields tends to be below average, with some colonies consistently experiencing high rates of complete nest failure. One possible hypothesis for factors contributing to poor productivity near rice fields is contamination by environmental pollutants. The red swamp crayfish *Procambarus clarkii*, a main prey species in rice fields is known to bio-accumulate heavy metals. White storks also forage extensively year round on landfill, which is also likely to increase body burdens of trace elements. For females, egg laying is an important route for the excretion of heavy metals, enabling body concentrations to be evaluated non-invasively.

This study compared differences in the distribution of 4 heavy metal species in 167 egg shells from 19 colonies in key foraging habitats: landfill, rice field and cork oak savannah (control colonies), to ascertain if rice field colonies had higher concentrations of key metal species known for their toxicity. These were mercury, lead, arsenic and cadmium. Content (yolk and albumen) and shell samples from the same egg were also analysed to ascertain whether concentrations deposited in shell was representative of the contamination experienced by the developing chick.

Results showed that, in this species, arsenic preferentially deposit in egg content, whilst there was no difference in the distribution of cadmium, mercury and lead between the shell and content. This means cadmium, mercury and lead in egg shell are representative of body burdens at the time of egg laying. None of the metal species were found in concentrations that were cause for concern. Additionally, eggs from nests that are in close proximity to landfill habitat (where productivity is good) were the most contaminated meaning it is unlikely that rice field colonies are suffering reduced productivity as a result of contamination by trace metals.

## 5.1. Introduction

Habitat quality and the availability of food resources are known to impact on breeding success (Martin 1987, Newton 1998), however, increasingly the quality of food resources is being impacted by anthropogenic activities. It is widely known that environmental pollutants, such as organo-chlorates, persistent organic pollutants (POPs), flame retardants and trace elements including heavy metals, can have negative effects on the population dynamics of local species (Gómez-Ramírez *et al* 2012, Casa-Resino *et al* 2015, De Luca-Abbott *et al* 2001). Contaminants bio-accumulate up the trophic levels and concentrate in apex predators, making them particularly susceptible to negative impacts (Bosveld *et al* 1995). Impacts include behavioural alteration, increases in stress hormones (Casa-Resino *et al* 2015) and reduced physical condition (with consequences for breeding fitness), reduced survival and lower breeding success (De Luca-Abbott *et al* 2001, Connell *et al* 2003). Breeding success can be affected in a variety of ways including reduced clutch size, egg shell thinning, egg hatchability, as well as impacts on egg and chick development (Custer 2000, Erwin and Custer 2000). Mercury, lead, cadmium and arsenic, normally derived from industry and smelting, are individually highly toxic and are widely monitored in breeding birds, (eg Golden *et al* 2003, Lam et al 2004, Ayas 2007). Synergistic and antagonistic interactions between heavy metals means that the combined impact of metals may be greater than their individual toxicity (Pain *et al* 1998, Baos *et al* 2006). This is known to make individuals more susceptible to disease and can increase the impact of environmental pressures, such as bouts of cold weather (De Luca-Abbott *et al* 2001).

In Southern Europe, rice fields are important foraging habitat for many avian species (Lourenco and Piersma 2009, Toral *et al* 2012), including the white stork. This species is becoming increasingly sedentary on the Iberian Peninsula and is known to utilize rice fields extensively throughout the year. However, recent analysis of 8 years of breeding success data in white stork colonies in Portugal has indicated that colonies close (within 4 km) to rice fields have below average productivity in comparison to other habitats (Chapter 4). In particular, some colonies consistently experienced high rates of complete nest failures, where monitored breeding pairs failed to raise any chicks.

A possible explanatory hypothesis may be that some rice fields experience environmental contamination from trace elements, such as heavy metals, and this is impacting productivity. Rice field colonies in the estuaries of the River Sado and Tagus in south-western Portugal are located close to areas of heavy industry associated with the capital, Lisbon, and its hinterland. Historically, potentially hazardous industrial emissions were less regulated which may have resulted in the deposition of industrial contaminants in areas that are now rice field. Wading birds are known to be particularly susceptible to contamination because they ingest sediment in large quantities as they forage (Pain *et al* 1998). Furthermore, in rice fields, white storks forage intensively on the abundant, invasive red swamp crayfish, which they feed to their chicks in large quantities during the breeding season (Negro *et al* 2000, Correia 2001). Crayfish are an abundant food source most of the year, but it is known to accumulate pollutants and heavy metals in its tissues and organs and transmit them up the trophic levels (Alikhan *et al* 1990, Geiger *et al* 2005, Alcorlo *et al* 2006), impacting particularly on the species at the apex of the food chain. The white stork, an apex predator, is therefore an indicator species for overall level of habitat contamination. Data from this study could be of interest to studies of other species who intensively use rice fields.

White storks also forage extensively on landfill, which likely influences heavy metal concentrations in eggs and chicks in nests breeding close to landfill (Casa-Resino *et al* 2014), since birds with nests located close to landfill sites tend to use landfill sites with high frequency throughout the year (Chapter 2). However, breeding success near landfills in the study area was high overall, suggesting minimal impact, which could be used as a bench mark comparison with rice fields.

Evaluation of levels of heavy metals in eggshell is a widely recognized, non-invasive monitoring method (Connell *et al* 2003, Evers *et al* 2003, Ikemoto *et al* 2005, Brasso *et al* 2012), and egg-laying is an important contaminant excretion method in females (Burger 1994). The levels of heavy metals, for example mercury, in eggshells are known to correlate with female blood concentrations, as well as concentrations in prey (Evers *et al* 2003). This means values reported in egg shells should reflect levels at time of breeding and indicate levels the developing chick is exposed to. Deposition in egg material is particularly important for this species because moult slows with age (van den Bossche 2002). This means in some years individuals do not replace any feathers, thereby being unable to deposit contaminants into feathers, another well-known excretion route.

The aims of this study were: 1) to compare the detectability of the metal species in the content and shell of white stork eggs, and 2) to compare the distribution of various metals in eggshells from rice fields, landfill and control colonies to ascertain if heavy metal contamination could be impacting productivity in white stork colonies located near rice fields.

## 5.2. Methods

### 5.2.1. Sample collection

During the breeding season of 2013, the area below nests in 19 colonies was frequently checked for egg shell and whole, unhatched eggs. Whole eggs may be expelled from the nest as a result of nest ownership changes, or because they failed to hatch. A mean (± SE) of 8.4 ± 0.5 eggshells per colony were collected. Shell and content samples were stored in individually labelled, sealed plastic bags until analysis.

The location of the 19 colonies is depicted in Figure 5.1 and sample numbers are shown in Table 1. The frequency with which individual white storks visit landfill is dependent on the distance between the nest and landfill site (Chapter 2), so landfill colonies were selected to be as close to landfill sites as possible (max distance: 6.7 km, mean ± SE: 4.1 ± 1.3 km). Many stork colonies are located immediately beside rice fields, thus rice field habitat forms a significant proportion of the foraging habitat immediately around the nest. Where possible, egg material was gathered from the same rice field colonies monitored for productivity (7 of the 9 rice field-only colonies, Chapter 4). This was to ensure that colonies with particularly low breeding success were targeted. Unfortunately, one colony was located on pylons over flooded rice fields and another was on urban roof tops, so no egg material was recovered. Three alternative colonies immediately beside rice fields were included as alternatives. Rice colonies were located a mean (± SE) of 0.1 km ± 0.1 from rice field and a minimum distance from landfill of 38.1 km (mean ± SE: 55.6 km ± 3.9).

Control colonies were located in extensive areas of cork oak savanna, a traditional, low intensity management system of cattle grazing and cork oak trees with some small crop fields on rotation cycles. This habitat was considered likely to be relatively uncontaminated by industrial pollutants. Control colonies were located a mean (± SE) distance of 48.0 km ± 8.3 from landfill and 35.7 km ± 5.6 from the nearest rice field.

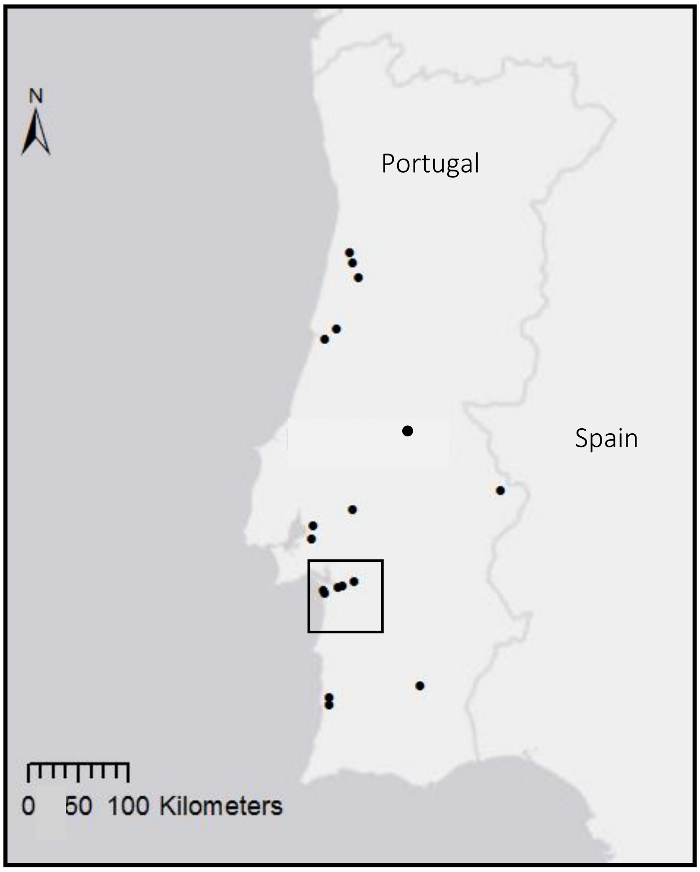

**Figure 5.1** *Location of colonies where egg samples were collected in Portugal. The area inside the box is the Sado Estuary, where productivity in rice fields Is particularly low.*

### 5.2.2. Laboratory analysis

Prior to analysis egg content was freeze dried to remove moisture content. Shell was cleaned of surface debris using distilled water and acetone, then dried in a fume hood for a minimum of 48 hours. Each 0.5g sample was acid digested with a Milestone Ethos closed vessel microwave acid digestion system using 10mls of ultra-pure nitric acid (65%). Afterwards, the sample volume was raised to 15ml using ultra-pure water (Mili-Q).

Shell and content samples were analysed for heavy metals (Cd, Pb, Hg and As) by ICP-MS (Internally coupled plasma mass spectrometry). 1ml of sample was diluted with 9ml of Mili-Q and spiked with 1m of Rhodium, the internal standard. Samples were analysed in batches of 20 with 3 Mili-Q blanks, a blank containing 1ml of rhodium and 5 standards between them. The 5 standards each contained 1ml of rhodium then stepped volumes (0.2, 0.4, 0.6, 0.8 and 1ml) of reference solution made up to 10mls with Mili-Q. The reference solution was mixed separately and contained known quantities of single element standards of Cd, Pb, Hg and As from CPI International.

Due to suspected interference from alkali metal oxides causing high deposition on the cones at the ionization stage, samples and standards were diluted again as follows. 1ml of sample diluted as above was made up to 10ml with a further 1ml of rhodium and 8ml of Mili-Q. Reported concentrations are blank corrected.

**Table 5.1** *Number of colonies and egg shell samples per habitat type.*

| | Habitat type | | | |
|---|---|---|---|---|
| | **Landfill** | **Rice field** | **Control** | **Total** |
| **Number of colonies** | 3 | 10 | 6 | 19 |
| **Total shell samples** | 25 | 81 | 61 | 167 |

## 5.3. Statistical analysis

Concentration data were not normally distributed. Wilcoxon signed rank tests were carried out to compare differences in metal concentrations between egg content and shell. Kruskal-Wallis tests were conducted to investigate significant differences in trace element concentration between the three habitat categories.

## 5.4. Results

### 5.4.1. Deposition site of metal species in egg and content

In the white stork, arsenic preferentially deposit in the contents (Z= -3.054, p= 0.002), meaning that levels reported from eggshells may be significantly lower than those the developing chick was exposed to. There was no statistical difference in deposition location (shell or content) in cadmium (Z= -1.485, p= 0.137), mercury (z= -314, p= 0.753) or lead (Z= -0.142, p= 0.887) meaning the levels reported in egg shell are likely similar to levels of exposure of the developing chick (Figure 5.2).

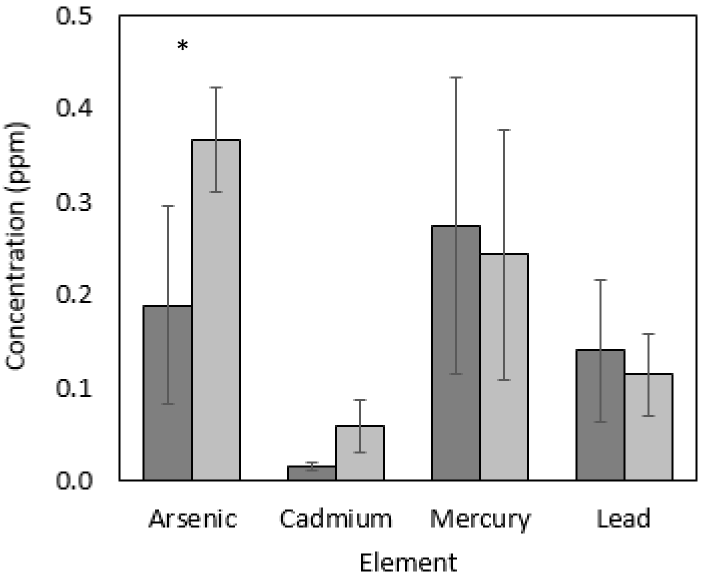

**Figure 5.2** *The mean (± SE) distribution and concentration of metal species in egg shell (dark bars) and egg contents (grey bars) from 18 whole eggs found below white stork nests. Statistically significant differences are *(p <0.05) and ***(p <0.001).*

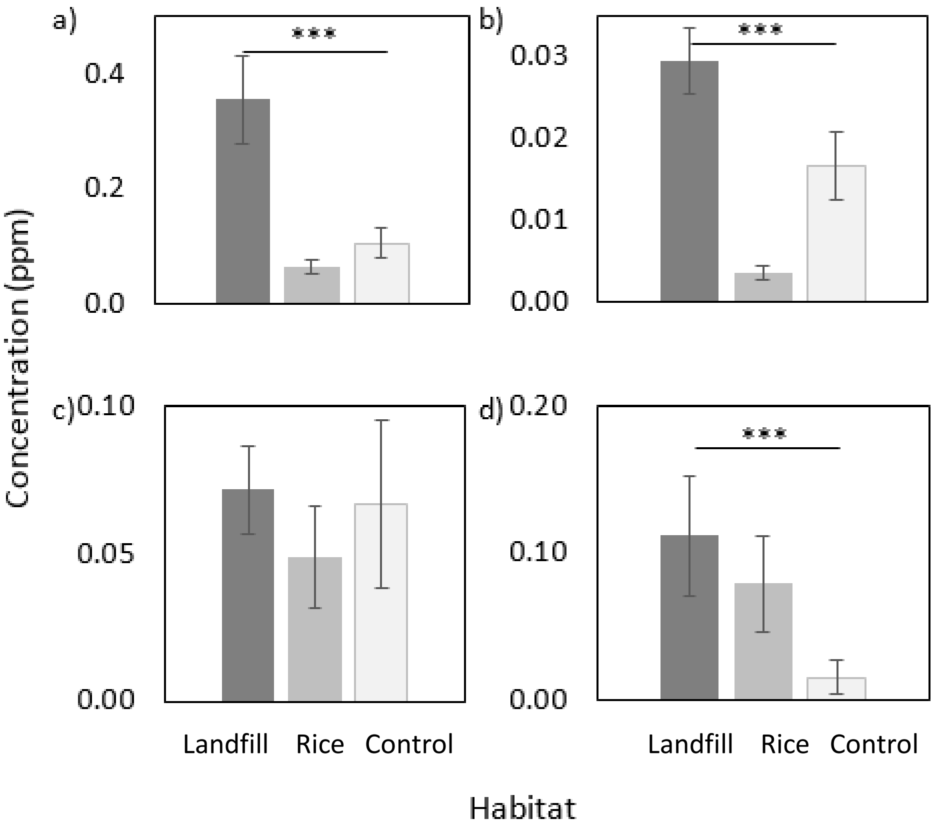

**Figure 5.3** *Mean (± SE) concentration of a) lead, b) cadmium, c) arsenic and d) mercury from eggshell sampled from colonies in 3 different foraging habitats. Colonies in landfill habitat are represented as dark bars (n=3 colonies), colonies near rice field (n=10) are grey bars and control colonies located in arable and cork oak habitats (n=6) are shown as pale bars. Statistically significant differences are *(p <0.05) and ***(p <0.001).*

### 5.4.2. The distribution and concentration of various metals in different habitats

The values for each metal ranged from below detection limits (all metals) to 1.66 ppm (arsenic), 0.16 ppm (cadmium), 1.49 ppm (lead) and 1.76 ppm (mercury). Levels of lead, cadmium and arsenic were lowest in rice field colonies of all the habitats (Figure 5.3). Levels of mercury in rice field colonies were significantly lower than quantities present in landfill. Landfill is the largest source of all metals in this study population and was statistically different to the concentrations found in rice fields (As: Z= -2.424, p= 0.015, Cd: Z= -6.895, p= <0.05, Hg: Z= -2.714, p= 0.007, Pb: Z= -4.574, p= <0.005).

## 5.5. Discussion and Conclusion

Determination of metal concentrations from eggshells is a widely used monitoring tool for assessing and monitoring environmental contaminants. Individually, lead, cadmium, arsenic and mercury are all highly toxic. In this study, in all habitats (Figure 5.3), abundancies of all 4 metals were equivalent to, or below, background levels reported in white stork eggs in Spain (Hernandez *et al* 1988) and also below levels reported in eggshells of species with similar ecologies, such as grey heron (Ayas 2007). Thus, in normal breeding years, the cause of low productivity in rice fields is unlikely to be related to the individual impact of any of the four heavy metals species investigated by this analysis.

Arsenic preferentially deposits in egg content in this species (Figure 5.2). This means that values recorded in shell are not representative of true body values. Overall, concentrations of all four elements was highest in landfills, which was not unexpected. As productivity is known to be good near landfills (Tortosa *et al* 2002, Massemin-Challet *et al* 2006, Chapter 4) this further indicates that the four metals were not present in abundances that are cause for concern. The closure of open landfills will reduce body burdens of trace metals in the white stork population.

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# Chapter 6

# Migratory status of lesser kestrels (*Falco naumanni*) in Iberia from carbon isotopes and identification of juvenile females using deuterium enrichment

To be submitted as:

Gilbert, N.I., Marca, A., Catry, I., Gilroy, J. Bustamente, J. and Franco, A.M.A., Migratory status of lesser kestrels (*Falco naumanni*) in Iberia from carbon isotopes and identification of juvenile females using deuterium enrichment.

## Abstract

Several previously wholly migratory species recently established resident populations in Europe. The ability to distinguish migratory from non-migratory individuals within partially migrant populations is essential to understand the consequences of changing migratory behaviour. This study uses stable isotopes of carbon, hydrogen and nitrogen from feather samples to determine migratory status of individuals from two populations of lesser kestrels *Falco naumanni*, a migratory population in Portugal and a partially migratory population in Spain.

Breeding and post breeding (winter) grown primary feathers were collected from 233 birds with unknown migratory status. Control breeding area feathers were sampled from chicks and juvenile males. The African wintering locations were known from 6 birds tracked with geolocators. Unexpectedly, the post breeding grown feathers of 3 tagged individuals showed an Iberian isotopic signal suggesting flexibility in timing of moult, with some birds completing moult before or immediately after migration. Carbon isotopes could therefore only distinguish migrants who moulted in Africa (82.1% of birds from the wholly migratory population, 42% from the partially migrant population). Deuterium ratios from winter-grown feathers were too variable to assign migratory status and there was no difference in nitrogen values between adult breeding and winter grown feathers in either population.

All adult primary feathers grown during breeding were highly enriched in deuterium compared to chick and juvenile feathers, possibly due to increased stress and evaporative cooling associated with breeding effort. Differences in enrichment were used to identify non-breeding (likely juvenile) females (6% of the migratory population, 5.3% of the partially migrant population). This novel use of deuterium potentially has applications for other studies where there is a requirement to identify the age or breeding status of individuals. Nitrogen isotopic ratios provided confirmation of the robustness of deuterium in identifying likely juveniles, but only in the migratory population.

## 6.1. Introduction

Partial migration, where some individuals within a population migrate whilst others remain in the breeding area, occurs frequently across diverse taxa, including fish, insects, mammals and birds (Chapman *et al* 2011, Chapman *et al* 2011b). Comparing migrants and non-migrants within the same population offers a unique opportunity to understand the causes and consequences of migratory decisions, which will improve our ability to predict how species may respond to future climatic and environmental change. Relatively few studies have compared individuals with contrasting migratory strategies (Chapman *et al* 2011a) and to do this it is important to be able to confidently distinguish a resident individual from a migrant.

Birds are good study species to understand the causes and consequences of partial migration due to a diversity of techniques that can be used to mark individuals. In populations with a high number of marked individuals, it may be possible to monitor presence/absence in the breeding grounds during the winter. However, this is labour intensive and resident individuals wintering even a short distance away from the breeding colonies may go undetected. Furthermore, ring recoveries from sub-Saharan Africa are notoriously low (Chamberlain *et al* 2000, Reichlin *et al* 2013). Data loggers and geolocators are increasingly high-resolution, light weight and low-cost enabling deployment on a large sample of individuals and ever-smaller species (Stutchbury *et al* 2009, Tottrup *et al* 2012). However, geolocators usually require recapture of marked individuals to acquire the data thus cannot be used for species with low site fidelity or low year on year survival (Bachler *et al* 2010, Hahn *et al* 2013). A non-invasive, quick and low-cost solution for separating migrants from non-migrants is the use of stable isotopes of hydrogen (deuterium ($\delta$D)), carbon ($\delta^{13}$C) and nitrogen ($\delta^{15}$N) and contrast the isotopic signatures of feathers grown in the breeding and non-breeding season.

Stable isotope analysis relies on the predictable geographic variation in ratios of abundance of the heavy to light isotopic species of an element. The isotopic signature of the local food web is taken up by all species through diet and drinking water and is metabolically inert after synthesis in keratinous body tissues, including feathers (Hobson 1999). These ratios are retained when a bird migrates to a new, isotopically distinct area which means inferences can be made about the location where the feather was grown. Therefore, provided diet is constant and moult sequence is understood sufficiently to confidently select feathers representative of the breeding area wintering area in the same individual, it should be possible to separate migrants from non-migrants by comparing the difference in isotopic ratios between the two feather samples. Using this method, the migratory strategy of all individuals in a study population can be investigated, not just ringed individuals, or surviving birds tagged the previous year.

Stable isotope analysis is now a well-established tool for assessing migratory connectivity and assigning geographic wintering areas to long-distance migratory birds (Rubenstein and Hobson 2004, Bowen *et al* 2005). In areas of strong isotopic gradients, isotopes have also been successfully utilized to identify resident from migratory individuals (eg Hegemann *et al* 2015). However, it has not been assessed if stable isotopes can distinguish individual migratory strategy in areas of low isotopic variance, such as between southern Europe and sub-Saharan Africa. This is particularly relevant because many previously wholly migratory species recently established resident populations in Iberia (Atlas Team 2008) making this a fascinating region for migration studies. This study assesses the ability of stable isotope investigations to infer migratory status between southern Europe and the Sahel region of sub-Saharan Africa. Furthermore, to date the majority of isotopic information from the Sahel is derived almost entirely from studies of passerines (eg Yohannes *et al* 2007, Pain *et al* 2004, Oppel *et al* 2011) so there is a conspicuous lack of isotopic information from other bird groups, particularly raptors. In order to be able to correctly interpret isotope data from raptor species, it is essential to have full understanding of mechanisms influencing isotopic ratios in tissues and how these differ from passerines. Isotopic data of known origin is an important tool in the conservation of Afro-Palearctic raptor species, many (51% Kirby *et al* 2008) of which are experiencing strong declines and are in poor conservation status. The Sahel region in particular poses potential threats to migratory raptors due to climatic and land-use changes and other anthropogenic impacts (Limiñana *et al* 2012), so isotopic data from this region is especially welcome.

Most Afro-Palearctic studies find carbon and nitrogen to be the most informative (Hobson *et al* 2012a). Carbon isotopic analysis can distinguish between feathers grown in a predominantly $C_3$ or $C_4$ plant photosynthetic pathway habitat (Still *et al* 2003, Still and Powell 2010). This has enabled both habitat selection by migrants (therefore likely wintering region) and migratory connectivity between populations to be established (eg Chamberlain *et al* 2000, Yohannes *et al* 2007, Evans *et al* 2012, Morrison *et al* 2013). Gradients are strong between Africa (predominantly $C_4$) and Iberia (predominantly $C_3$) meaning carbon was expected to be the most informative isotope in this study. Nitrogen fractionation depends on moisture availability and trophic level and previous studies have used differences in nitrogen, in conjunction with carbon, to infer distinct population segregation in migratory birds in the wintering area (eg Møller and Hobson 2004, Bensch *et al* 2006). Nitrogen may therefore be useful as an indicator of migratory status if there are noticeable differences in diet (trophic level changes) between the breeding and winter areas.

Several studies have assessed the value of using hydrogen to differentiate breeding from wintering areas. Hydrogen gradients are strongly influenced by the isotopic composition of precipitation which becomes continuously more isotopically depleted moving from low to high latitudes and with increasing continentality (Dansgaard 1964). Thus, many studies, particularly in the Americas where this gradient is well established, have found strong links between the hydrogen composition of feathers and geographic origin (Hobson *et al* 2012b). However, there is little variation in hydrogen isotopic composition between Iberia and the Sahel (4-6‰) which may render the two regions difficult to distinguish (IAEA/WMO 2015). Additionally, the scarcity of precipitation in some regions, paucity of sampling stations, high variability between feathers from the same area and lack of ground-truthed feather samples means hydrogen gradients are low resolution and inadequately described (Bowen *et al* 2005, Gutiérrez-Expósito *et al* 2015) leading some studies to conclude that hydrogen is not particularly useful for assigning geographic area in Africa (Pain *et al* 2004, Møller and Hobson 2004, Meehan *et al* 2003, Oppel *et al* 2011). The few that found deuterium meaningful were at broad geographic scale (eg Reichlin *et al* 2010, Reichlin *et al* 2013). By combining multiple stable isotopes with data from geolocators was hoped to enable this study to overcome these issues.

Deuterium interpretation is also confounded by a lack of knowledge concerning the causes (physiological and environmental) and extent of variance in diet-tissue fractionation factors within individuals and across age classes (Wunder and Norris 2008, Betini *et al* 2009). Unlike carbon and nitrogen, which are derived almost entirely from food, body water is derived from many sources (drinking water and from prey), each source pool with its own individual isotopic fractionation conditions. Body water is also lost by many sources (for example respiration and sweating) and loss rates vary according to physical effort, body size and individual metabolic rate. This may contribute to explaining why some feather - precipitation isoscapes have low goodness of fit. Some studies have found significant enrichment in deuterium in adult feathers compared to chick feathers from the same species grown in the same area (Meehan *et al* 2003, Smith and Dufty 2005, Lott *et al* 2006, Powell and Hobson 2006, Hobson *et al* 2009, Greenwood and Dawson 2011), and these far exceeded levels expected in passerines and predicted by precipitation. It was therefore not certain in this study if deuterium ratios would be predominantly influenced by precipitation or another factors.

This study aims firstly to determine which isotope, or combination of isotopes, best distinguishes Iberia from the Sahel using a partially migratory species, the lesser kestrel, as a case study; and secondly to use isotopic ratios from a breeding and winter grown feather from the same individual to estimate the percentage of residents and migrants in two populations, a migratory population in Portugal, where there are presumed to be no/few resident birds, and a partially migratory population in Spain. However, in view of the differences in deuterium values found between age classes during the course of this study, a third aim became to test whether deuterium enrichment could be used to identify non-breeding (likely juvenile) birds. This is particularly useful in the lesser kestrel because juvenile females (with no migratory status) are not easily distinguished from adult females by plumage.

## 6.2. Methods

### 6.2.1. Study species and study site

The lesser kestrel *Falco naumanni*, was considered an ideal study species to address the aims of this study because it is thought to be wholly migratory in Portugal with no individuals staying in breeding colonies during the post-breeding season, but is known to be partially migratory in Spain, where colonies have both migratory and resident individuals (Negro *et al* 1991). We sampled individuals from 5 colonies from the migratory (M) population, Castro Verde, Portugal (37.690515, -7.997410) and from 7 colonies from the partially migratory (PM) population, Gerena, Spain (37.528283, -6.154926). The sampled populations, with different migratory strategies, exist in close proximity to each other in an isotopically comparable region (IAEA/WMO (2015), allowing robust comparison between them.

Details of migratory routes and timing of migration was known for a subsample of birds from the migratory Portuguese population, from light level geolocators deployed by a previous study (Catry *et al* 2011a). Feather samples (P9, T3) were collected from the individuals with geolocators enabling the verification of the wintering area and contributing to the much needed isotopic data variation in feather isotopes of a raptor species in the Sahel.

### 6.2.2. Feather sampling

One or more feathers samples were collected from a total of 233 birds over 3 different years in Portugal (79 birds, years 2009, 2013) and Spain (160 birds, 2013/14) from birds caught in nest boxes in breeding colonies. Adult males are easily recognised, juvenile and adult females have indistinguishable plumage. Based on previous knowledge of lesser kestrel moult patterns (Cramp and Simmons 1977), the primary P9 was considered the best feather to characterise the wintering area.

A total of 79 birds were sampled from breeding colonies in the migratory population in Portugal, where no individuals remain during the winter. In 2009 the winter grown feather P9 and the breeding season grown tail feather T3 were sampled from 32 birds, including 6 individuals whose sub-Saharan African wintering location was known from light level geolocators (see Catry *et al* 2011a). During the first batches of isotopic analysis, the T3 feathers were realised to be unreliable as an indicator of the breeding area due to high isotopic variability in carbon values. They were not included in the results. In the 2013 breeding season an additional 47 Portuguese birds were sampled when P9 (winter grown) and P3 (breeding grown) feather samples were collected. In total 28 individuals were identified from plumage as adult males, 51 were females of unknown age.

To isotopically characterise the Iberian breeding area, and to establish the base level for deuterium in non-breeding birds, breast feathers were collected from 25 chicks from the migratory (Portuguese) colonies, in 2013. Analysis of all primary feathers from dead birds (see below) showed that the P3 feather is grown at the end of the breeding season when some birds may already be migrating. This indicated that P4 would in fact be a better feather to characterise the breeding area. Consequently, during the 2013 winter and the 2014 breeding season, we sampled P4 and P9 feathers from 160 birds in breeding colonies from the partially migratory (Spanish) population known to have both resident and migrant individuals. Approximately 25-30% of individuals are resident in these colonies, and it is likely that some individuals overwinter in Iberia away from their breeding colonies (Negro *et al* 1991, author's unpublished data). Of birds sampled from Spain, 79 were adult males, 13 were known from rings to be adult females and 68 were females of unknown age. Breeding season P4 feathers were also taken from 40 birds known from plumage to be juvenile males. Feathers were stored individually in labelled sample bags until analysis. All 3 isotopes were analysed from Portuguese samples, whilst for Spanish, every isotope was analysed in a subset of feathers.

Moult sequence and consistency of isotopic variance across the wing were established by sampling all primary feathers (P1:10) from 9 lesser kestrels (*n*= 6 female, *n*= 3 male) found dead in 2013, using stable isotopes of $\delta^{13}C$, $\delta^{15}N$ and $\delta D$. Three (2 female, 1 male) were from the migratory Portuguese colonies and 5 were from the partially migratory Spanish colonies and included a known first year female.

### 6.2.3. Stable isotope analysis

A sample of 1.5 x 1.5cm of material was clipped consistently from the same distal portion of the feather to avoid any systematic differences in isotopic ratios within the feather that have been noted in some raptor species (Smith *et al* 2008). They were washed in a 2:1 chloroform:methanol solution to remove surface oils and dirt, then dried under the fume hood for 48 hours. Each sample (excluding rachis) was cut as finely as possible, cutting parallel to the rachis to capture as much isotopic variation as possible in each fragment. 0.5mg of sample were weighed and tightly packed in to tin capsules. To account for continuous exchange with atmospheric moisture, the standards and replicate samples intended for Deuterium analysis were loosely covered and allowed to equilibrate at room temperature with the ambient laboratory air for a minimum of 72 hours before being scrunched closed.

All samples were analysed using a Thermo Finnigan Delta XP continuous-flow isotope ratio mass spectrometry at the Stable Isotope Laboratory (ENVSIL), University of East Anglia, UK. Carbon and nitrogen isotopic composition $\delta^{13}C$ and $\delta^{15}N$ were simultaneously analysed using a Costech Elemental Analyser on-line with a mass spectrometer. For hydrogen isotopic analysis, a Vecstar vertical furnace with a glass carbon packed pyrolysis column was used on-line with the same mass spectrometer. Two newly developed international keratin standards from the US Geological Survey (USGS 42 and USGS 43; Coplen and Qi 2012) and an inter-comparison material independently developed by the Doñana Biological Station, Spain (LIE-PA2, Razorbill feather; Alvarez 2012) were analysed at the start and end of batches to ensure consistency between batches. Some samples had repeated analysis in different batches as an additional consistency check. A triplicate of the USGS43 was analysed after every 12 unknown samples to account for instrument drift quantification. Due to faulty carrier gas flow affecting the nitrogen portion of the analysis, some batches of the Spanish feathers had to be remeasured. In some cases there was insufficient sample remaining so carbon data only exists for these feathers.

Assuming the proportion of exchangeable hydrogen in feather keratin was 20% (Wassenaar and Hobson 2000) an isotopic scale stretch correction was applied to all samples using the known non-exchangeable fractions of USGS 42 (-78.5‰), USGS 43 (-50.3‰) and Razorbill (20.8‰).

Stable isotope compositions are reported in δ notation in parts per thousand (‰) deviation from the international standards for Carbon (Pee Dee Belemnite (PDB)), nitrogen (Atmospheric Nitrogen (Air)) and for hydrogen (Vienna Standard Mean Ocean Water (VSMOW)). This is defined by the equation: δsample=([Rsample/Rstandard]-1)x1000 where δsample is either $^{13}$C, $^{15}$N or $^{2}$H respectively, relative to the standard and R is the ratio of the heavy and light isotopes ($^{13}$C/$^{12}$C, $^{15}$N/$^{14}$N or $^{2}$H/$^{1}$H respectively) in both the sample and reference material. Measurement precision for δ13C and δ15N was estimated to be ≤0.2‰ and for Hydrogen was ≤2.0‰

### 6.2.4. Assessing the effects of body size and heat stress on feather hydrogen

Heat loss efficiency, and the degree of deuterium enrichment due to water loss through the skin, may be linked to body size so simultaneously with feather harvest, birds of unknown migratory status were sexed and wing chord length, (a proxy for body size) was measured. Weight was also measured but was not included in this study because it can fluctuate according to time since last feeding, internal egg development and other factors, so a single measure of weight was not considered a sufficiently reliable indicator of body size.

Evaluation of the impacts on feather deuterium ($\delta D_f$) of heat stress from prolonged exposure to high temperatures in artificial nest boxes was possible for 6 of the 25 Portuguese chicks, each from a different brood. This came about because temperature was being recorded in a subset of nests by a different study and 6 nest boxes overlapped with birds in this study. Artificial nests have very different micro-climates depending on the construction material and aspect (compass orientation). Micro-T DS1922L temperature loggers recorded temperature inside nests and externally in the shade every 60 minutes. Three chicks were from the hottest nest type, wooden boxes, three were from cooler nests in concrete cavities in purpose-build breeding walls where temperatures remain similar to the ambient air. See Catry *et al* 2011b for further details of nest types and the impact of nest temperature on chick survival. Weight and wing chord length were measured at the time of feather sampling.

## 6.3. Statistical Analysis

### 6.3.1. Inter annual differences in isotopic ratios

Differences in isotopic ratios of $\delta^{13}C$, $\delta^{15}N$ and δD between years (2009 and 2013) and between migratory (Portuguese) and partially migratory (Spanish) populations were investigated using Mann-Whitney U tests.

### 6.3.2. Testing the evaporative cooling hypothesis

Linear regressions were used to assess the relationship between wing chord length, a proxy for body size, and deuterium enrichment in the breeding season grown feather (P3 and P4) in birds known to be adult. Mann-Whitney U tests were used to evaluate differences in deuterium enrichment between sexes in feathers grown at the beginning (P4) and end (P3) of the breeding season.

Linear regressions were carried out to examine the relationship between $\delta D_f$ and chick wing length (mm) and cumulative total temperature above $30^0C$ experienced prior to feather harvest. Our field observations suggest $30^0C$ is the mean temperature, taking in to account nest box type and compass orientation, at which chicks start to display signs of heat discomfort including panting and weight loss. Accumulated heat over $30^0C$ inside the nest box was quantified hourly using the formula used to calculate Growing Degree Days (GDD30). For each nest box, the hourly maximum temperature was subtracted from the threshold temperature of $30^0C$. Hourly values were then summed to make an accumulated total for each nest box. Only positive values (temperatures of $30^0C$ or more) contributed to the final total.

### 6.3.3. Assigning migratory status

Comparisons between feathers grown in the breeding and wintering areas were made using Mann-Whitney U tests and Kruskal-Wallis tests.

## 6.4. Results

### 6.4.1. Inter annual differences between feathers from different years

P9 winter feathers from Portugal collected over 2 years (2009 and 2013) were pooled as one sample. There was no inter annual difference between years in ratios of $\delta^{15}N$ (Mann-Whitney U= 695.00, p= 0.925) or $\delta^{2}H$ (U= 876.00, p= 0.791). There was an inter-annual difference in carbon means but not in the range of carbon values (Mann-Whitney U= 475.00, p= 0.016, mean ±SE 2009: -18.9‰ ± 0.6, 2013: -16.7‰ ± 0.7, Appendix 6.1).

### 6.4.2. Isotopic variance of feather isotopes from the wholly and partially migrant populations

*Carbon*

Carbon isotopic signatures enabled good characterisation of the breeding area. The range and mean values obtained did not differ between the two Iberian populations P3 (Migratory, M) (mean: -21.9‰ ± 0.1) and P4 (Partially Migratory, PM) (mean: -22.7 ‰ ± 0.05) (U= 459.00, p= 0.960), or between the chicks from the migratory population and the juvenile males from the partially migrant population (chick mean ±SE: -23.1‰ ±0.35, juvenile males: -23.0 ±0.12). The carbon signature of the breeding area was consistent across all groups analysed and displayed small value ranges, Figure 6.1a.

The carbon isotopic ratios of winter grown P9 feathers from both populations showed overlap with the breeding range values, with higher degree of overlap in the partially migratory population suggesting, as expected, a higher frequency of resident birds in the partially migratory population. Carbon values of the wholly migratory population (P9(M), mean: -17.6‰ ± 0.4) span a range of values from those similar to the Iberian breeding area (chicks) to the less negative values of the Sahel wintering area (isotopically different from the chicks and P3(M) feather). The mean from the partially migrant population P9(PM) feathers was (-21.2‰ ± 0.2), similar to isotopic ratios characteristic of Iberia. Migratory strategy could not be reliably identified from the carbon isotopic signature in feathers since, unexpectedly, the post-breeding

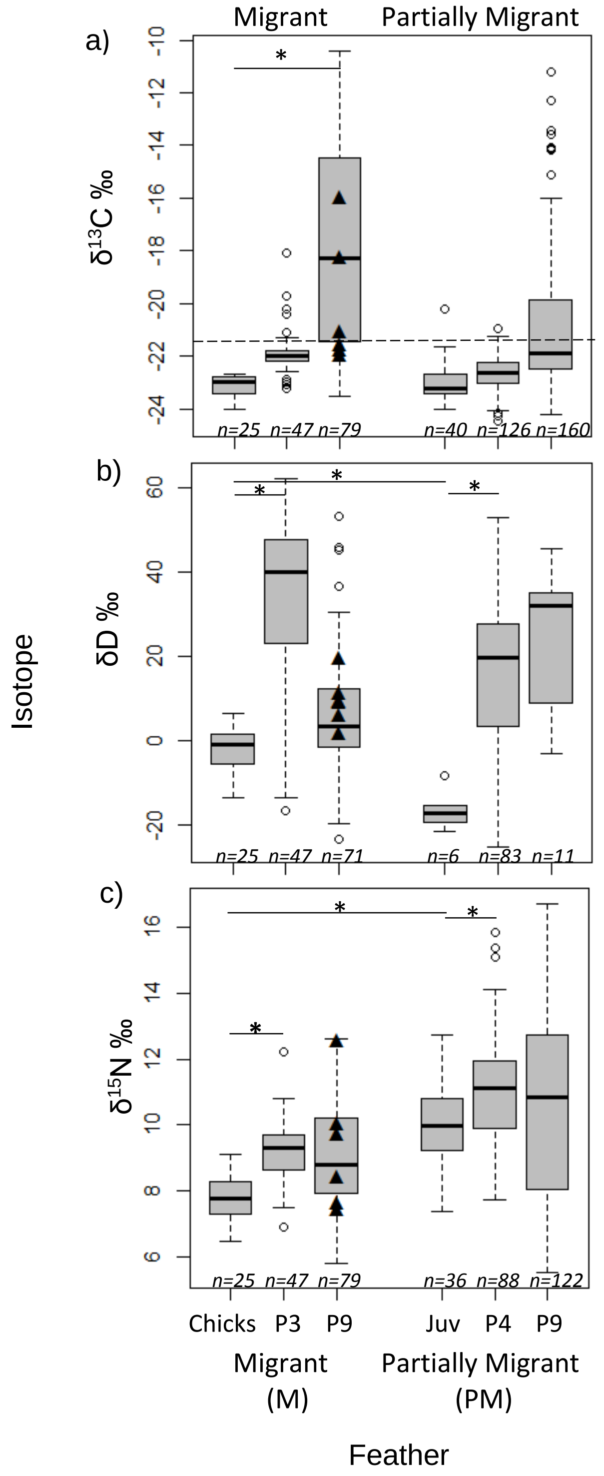

**Figure 6.1** *Isotopic compositions of a) carbon ($\delta^{13}C$ vs VDB), b) hydrogen ($\delta^{2}H$ vs VSMOW) and c) nitrogen ($\delta^{15}N$ vs AIR) of lesser kestrel young and adult primary (P3 and P4) feathers grown in Iberia and adult feather (primary P9) grown in Iberia or in the Sahel, Africa. Feathers (chicks, P3 and a subset of P9) derive from a wholly migratory (M) population from Portugal. Feathers (Juvenile males (juv), P4, and a subset of P9) are from a partially migrant (PM) population from Spain. The wintering*

*location of six adults from the migratory population were known from geolocators and values for the P9 feathers of tagged individuals are shown as triangles. The 95th percentile for carbon of the combined P3 and P4 feather is the threshold defining the breeding area and is represented as a dashed line in graph a). The total number of individuals in each sample are listed. Asterisks indicate significant differences between feather types.*

P9(M) carbon isotopic ratios of 3 of the 6 adults known, from geolocators, to have wintered in the Sahel overlapped with the range of values obtained for the breeding area P3(M) feather indicating flexibility in the timing of moult, with some individuals completing their moult in the breeding site or soon after arrival at the wintering areas.

*Deuterium*

Breeding area isotopic ratios determined by chicks and juvenile males indicate significant differences between the two populations (U= 3.00, p< 0.001, Figure 6.1b), chicks from the migratory population being more enriched. In both populations the mean of breeding season feathers from birds of unknown migratory status are significantly more enriched than the young birds (P3(M): 33.0‰ ± 3.2, P4(PM): 13.8‰ ± 2.01).

Hydrogen isotopic ratios of the migrant population show greater deuterium enrichment during the breeding period than during the post-breeding period. On average P3(M) values are higher than P9(M) (Figure 6.1b). Deuterium values of the post-breeding grown P9 feather are highly variable, the mean of the migratory population (P9(M): 5.9‰ ± 1.8) being less enriched than P3 feather and more similar to the chicks, whilst P9(PM) values (mean: 24.8‰ ± 5.1) are enriched and strongly disimilar to the juveniles (Figure 6.1b). The deuterium values of the winter P9(M) of the 6 tagged birds overlap with the chicks. Deuterium is therefore not useful for assigning migratory status.

*Nitrogen*

Nitrogen was not useful in determining migratory status since there was no isotopic difference between the values of breeding and winter grown primary feathers in either the partially migrant (P4(PM), P9(PM) feathers, Wilcoxon Rank Z= -1.848, p= 0.650) or migrant populations (P3(M), P9(M) feathers: Z=-0.577, p= 0.564, Figure 6.1c). There was a significant difference between young birds and breeding season feathers in both the migratory (chick and P3(M): U= 49, p<0.001) and partially migrant (juvenile males, P4(PM): U= 898, p= 0.002) populations. However, differences between the means were small, particularly in the partially migrant population (difference: 0.9‰) and there was considerable overlap in the value ranges between the P4(PM) (mean: 11.0‰, min: 7.7‰, max: 15.84‰) and juvenile males (mean: 10.1‰, min: 7.4 ‰, max: 12.7‰). There was a slightly larger difference in the means (1.5‰) in the Portuguese population (chick mean: 7.7‰, adult mean: 9.2‰). It was also noted that all feathers sampled from the migratory (Portuguese) population were more enriched in nitrogen than birds from the partially migrant (Spanish) population, Figure 6.1c.

### 6.4.3. Identification of long distance migrants using carbon

The breeding area was delimited for carbon by combining P3 and P4 feather values and taking the 95th percentile (-21.2‰). The 95th percentile was considered a robust value that would exclude the occasional replacement of feathers out of the normal sequence potentially leading to isotopic values outside the range expected for the breeding area. The 95th percentile provided a conservative estimate for the maximum isotopic value expected for Iberia and is represented as a dashed line on Figure 6.1a. Thus, 82.1% of individuals sampled from the migrant population were identified as migratory, including 3 of the 6 migratory tagged birds. In the partially migrant population only 42% of individuals were classified as migrant, (including 7 females known from rings to be adult). The majority (58%) of winter grown P9 feathers from the partially migratory colony were below this threshold and had Iberian breeding area values for carbon.

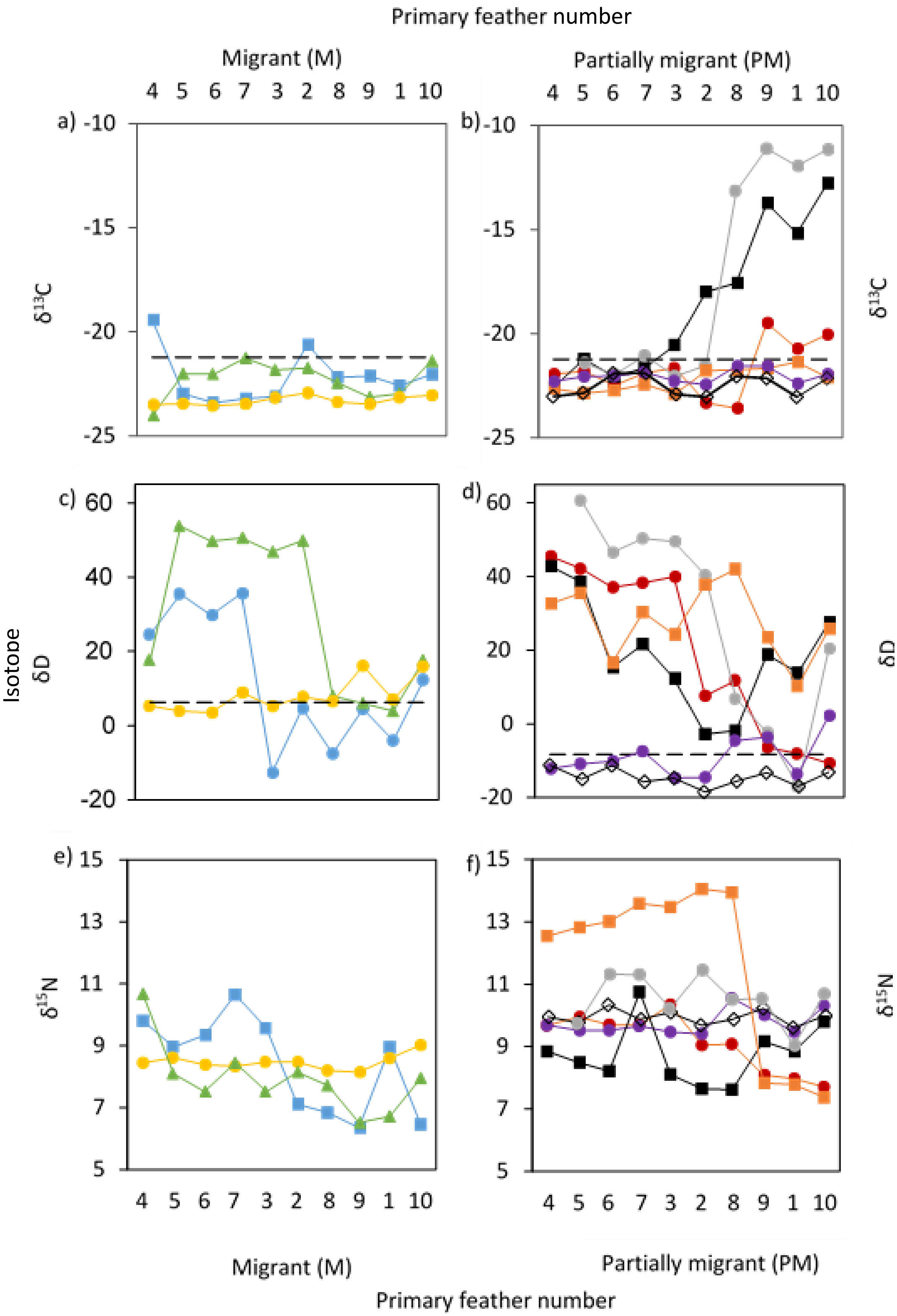

**Figure 6.2** *Isotopic compositions: $\delta^{13}C$ (a and b), $\delta^{2}H$ (c and d), and $\delta^{15}N$ (e and f) of primary feathers P1 to P10, ordered by moult sequence from the migrant and partially migrant lesser kestrel populations. Primaries P4-7 are known to be moulted in Iberia during breeding, the rest are moulted after in either Iberia or Africa. Each individual is represented by a different colour. Male birds (all adults) are squares, females (age*

*unknown) are circles, a first year female is identified by open diamonds and a female known from rings to be an adult is shown as triangles. In graphs a and b the dashed lines represent the 95th percentile threshold value defining the Iberian breeding area for carbon. In graphs c) and d) the baseline deuterium enrichment level is derived separately for each population from the 95th percentile of the young birds. Three birds show low isotopic variability in all isotopes, a female (depicted in yellow) from the migrant population, an adult male (purple) and juvenile female (open diamonds) from the partially migrant population.*

### 6.4.4. Consistency of moult and enrichment across primary feathers

#### 1) Moult

The moult sequence, according to the majority of the 9 dead birds and from field investigations, is in the sequence displayed in Figure 6.2 (P4, P5, P6, P7, P3, P2, P8, P9, P1, P10). Carbon and hydrogen isotopic compositions of the P4, P5, P6 and P7 feathers are consistent with values expected in the breeding area, therefore were grown during the breeding season. The remaining feathers are grown in the non-breeding period. Carbon data showed relatively little variance in order of moult of primary feathers between individuals, P4 being the first feather to moult during the breeding season for both males and females, whilst P3 is moulted at the end of breeding, or shortly after. However, our field observations during the breeding season have noted birds moulting 2 feathers simultaneously (for example P4 and P5, or P5 and P6). The remaining feathers are moulted post-breeding either in Iberia or in the sub-Saharan African wintering area. These results suggest that P9 is a good winter grown feather to contrast with isotopic values of the breeding area.

#### 2) Breeding season feather enrichment

*Characterising deuterium enrichment thresholds in young birds*

Due to significant differences in deuterium between the chicks (n=25, migrant population) and the juvenile males (n=6 partially migrant population), a threshold value of baseline enrichment in non-breeding, juvenile birds was created for each population using the maximum feather value (from Figure 6.1, chicks: 6.3‰, juvenile males: -8.3‰, threshold shown in Figure 6.2c and d). It was not considered necessary to take the 95th percentile value because both groups displayed a small value range and none of the young birds had ever migrated, so all feather values were considered representative of the breeding area. The majority of breeding season grown feathers P3 and P4 feathers are more enriched than these thresholds.

*Isotopic enrichment across all primary feathers*

For all birds, feathers grown in the breeding area (P4-7 and P3) showed low variance in carbon (Figure 6.2a and b) and values on or below the threshold limit defined for Iberia (shown as a dashed line).

Three individuals, likely juvenile birds still with chick grown feathers (1 known first year female (open diamonds) and 2 females of unknown age (depicted in yellow and purple, Figure 6.2), showed low isotopic variability across all feathers for all 3 isotopes and carbon and deuterium values consistent with young birds in all primary feathers. The remaining 6 birds (adult males and females of unknown age) showed strong, consistent enrichment in deuterium of all breeding season grown feathers in comparison to young birds (Figure 6.2 c and d). Enrichment between individuals was highly variable however, in this small sample, males tended to be less enriched than females across all breeding season grown feathers.

### 3) Winter grown feather enrichment

For carbon, the winter grown feathers of 2 individuals, P8 - P10 (female depicted in grey and the male depicted in black, Figure 6.2), have isotopic signatures consistent with the African wintering area ($\delta^{13}C_f \leq -15‰$). Surprisingly, all the remaining birds, including known adults from the migrant population, showed low carbon variance across all feathers and more enriched $^{13}C$ values consistent with Iberia (Figure 6.2a and b). This suggests that 7 of the 9 birds completed moult in Iberia rather than in the African wintering area. Hydrogen and nitrogen isotopic values of post-breeding grown feathers from the 2 migratory individuals were indistinguishable from the other birds (Figure 6.2d and f), which is consistent with the result that neither of these isotopic measurements can inform migratory status.

Hydrogen values of feathers moulted post breeding were highly variable but generally less deuterium enriched than early breeding season grown feathers and isotopically more similar to the young birds. This suggests an overall trend of decreasing feather enrichment across the moult as the breeding season progresses. However, an increase in enrichment between the P1 and P10 feathers in all birds, even the known juvenile, was also noted.

### 6.4.5. Effects of evaporative cooling on feather deuterium

*Chicks*

There was a significant positive relationship between feather deuterium and cumulative exposure to temperatures above $30^0$C (Figure 6.3a). The difference between the least enriched and most enriched chick feathers was 17.4‰. The maximum temperature experienced inside a nest box was 38.5C. There was an almost-significant, positive relationship between feather deuterium and wing length (figure 6.3b). However, there was no significant relationship between enrichment and the standardized residuals of wing length and GGD30 so it was unclear if body size or temperature is the main explanatory effect. There was no significant relationship between deuterium enrichment and wing length (F=(1,5)= 0.390, $R^2$= 0.072, p= 0.560 in the juvenile males from the partially migrant Spanish population.

*Adults*

There was no effect of sex on enrichment of the P4(PM) feather grown at the start of the breeding season (males *n*= 47, females *n*= 42, Mann-Whitney U=830.00, p= 0.197), or on the P3(M) grown at the end of the breeding season (males *n*= 15, females *n*= 32 Mann-Whitney U= 161.0, p= 0.071). There was also no relationship between deuterium values of breeding season grown feathers and wing chord length, a proxy for body size, in known adults in either population (P4(PM): (F(1,46)= 0.0,

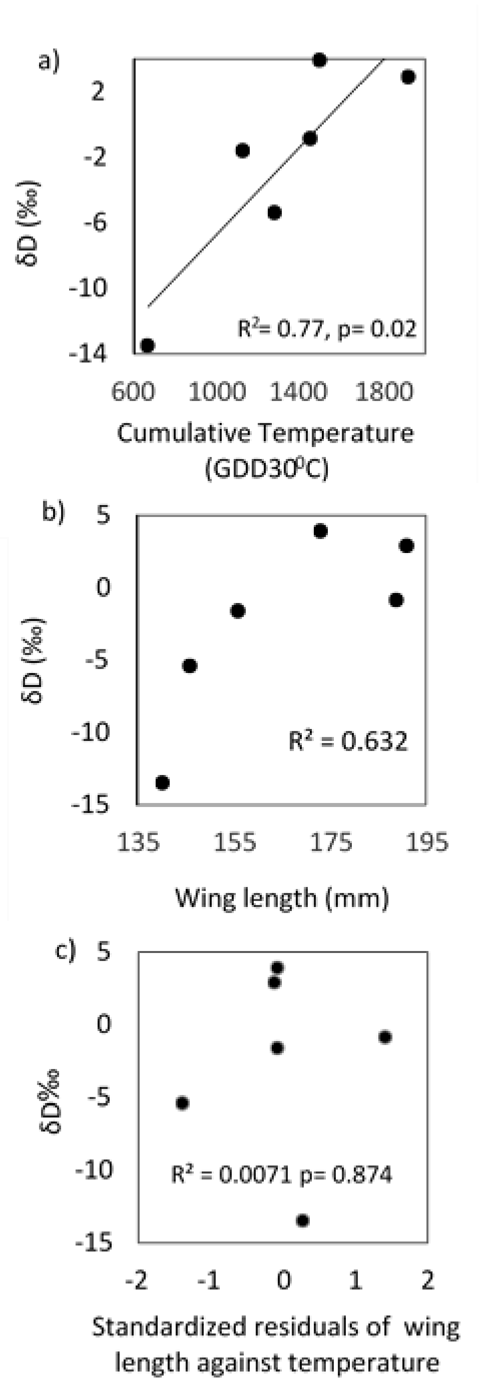

**Figure 6.3** *Hydrogen isotopic composition of chicks feathers in relation to a) cumulative hourly temperature experienced in the nest ≥30$^0$C, equivalent to Growing Degree Days above 30$^0$C ((GDD30) a measure of heat accumulation, b) wing chord length (a proxy for body size) and c) standardized residuals of the relationship between wing length and GDD30 for 6 chicks from the migrant population.*

p= 0.891, P3(M): (F(1,15)= 0.140, p= 0.983). Thus, there was no effect of any metric of body size on deuterium enrichment in adults.

### 6.4.6. Identification of non-breeding (juvenile) females using deuterium

All adult birds trapped in breeding colonies likely undergo a breeding attempt. Isotopic values of the majority of known adults, who underwent reproductive effort, are significantly higher in comparison to the young non-breeding birds who underwent no reproductive effort (migratory population: Mann Whitney U=103.00, p<0.001, partially migratory population: U=6.000, p <0.001, Figure 6.4). Females of unknown age, therefore unknown reproductive effort, overlap with both chick and adult feathers suggesting reproductive effort by some but not others. In the migratory population 11.9% of females of unknown age had P3(M) values below the baseline enrichment threshold delimited by the chicks, suggesting these individuals had no/little reproductive effort. In the partially migrant population 5.3% of female P4(PM) values showed no reproductive effort. A small percentage of birds known to be adults showed little enrichment and overlapped with the non-breeding values associated with young birds. This means, 7.9% of adults in the partially migrant population and 1.6% in the migratory population showed little or no evidence of reproductive effort.

### 6.4.7. Congruence between deuterium and nitrogen isotopes

In the migratory population there was no difference in nitrogen isotopic composition between the chicks and birds assigned juvenile status by using hydrogen isotopic composition (U= 49.00, p= 0.976), but there was a significant difference between birds assigned adult status and those assigned as juveniles (U= 80.000, p< 0.001) (Figure 6.5). In the partially migrant population there was too much overlap in the value ranges of known juveniles (min: 7.4‰, max: 12.7‰) and confirmed adults (min: 8.4, max: 15.8‰) to draw robust conclusions about unknown birds using nitrogen.

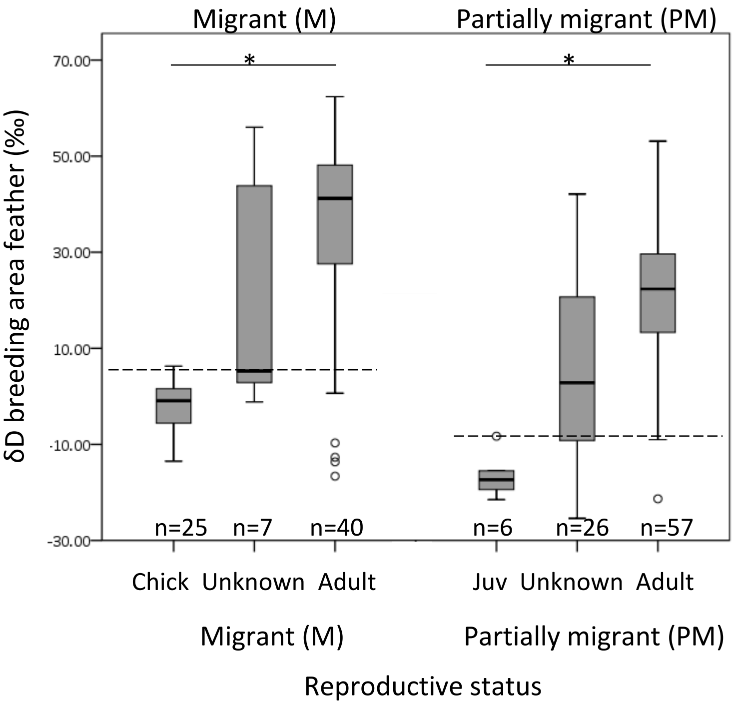

**Figure 6.4** *Reproductive effort gauged from deuterium enrichment of breeding season grown feathers from young birds (no reproductive effort), females of unknown reproductive status and confirmed adults (reproductive) from both the migrant (M) and partially migrant (PM) populations. The 95th percentile of the young non-breeding birds (dashed lines) are shown for both populations. Asterisks indicate statistically significant differences between young birds and adults.*

## 6.5. Discussion

### 6.5.1. Inter annual differences in isotopic ratios

Breeding season feathers from multiple years were directly comparable. All feathers came from the same breeding colonies and there were no significant changes in the local habitat during this time. Additionally, there was no isotopic difference in carbon between the chicks from Portugal and the juvenile males from Spain suggesting the carbon signal of Iberia is robustly characterised. Inter annual variance in the post breeding grown P9(M) was considered likely due to variance in

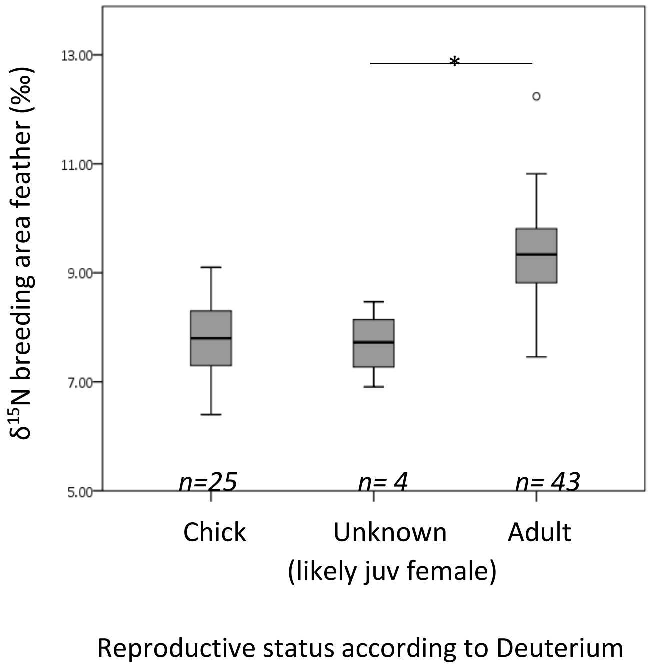

**Figure 6.5** *Nitrogen isotopic composition in feathers grown in the breeding season for the wholly migrant Portuguese population. Birds are defined as adult or juvenile according to their reproductive status based on the hydrogen isotopic composition. The asterisk indicates a statistically significant difference between juvenile females and reproductive adults*.

the proportion of birds completing moult in Iberia rather than after migration to sub-Saharan Africa. Tracking studies from both Spain and Portugal suggest birds are known to congregate in large winter roosts in Senegal, Mauritania and Mali (Rodriguez *et al* 2009, Catry *et al* 2011a, Limiñana *et al* 2012) in a very small latitudinal range of 4-17$^{0}$N making it unlikely that wintering location differed significantly between years. There was no difference in nitrogen values between winter and breeding season feathers, or between years in the winter grown feather. There was also no inter annual difference in hydrogen isotopic composition, however this may be due to the large range in values derived from the P9 feather.

### 6.5.2. Determining the migratory status of lesser kestrels between Iberia and Africa

#### 1) Carbon can only identify long distance migrants

In terms of migratory behaviour, the most informative isotope for this species is carbon,$^{13}$C, which shows clear differences between feathers grown in the Iberian breeding area and feathers grown post breeding, Figure 6.1. Chicks and juvenile males isotopically characterise the breeding area. The mean $\delta^{13}$C of the Portuguese chicks (-23.1‰ ± 0.35) is identical to the 3 passerine species average (-23.1‰ ± 0.35) generated in Portugal by Neto *et al* (2006). These are typical of values associated with a predominantly $C_3$ photosynthetic pathway habitat such as the grain crops and Mediterranean cork oak savannah grassland surrounding the breeding colonies (Aires *et al* 2008). The breeding season feathers of birds of unknown migratory status (P4(PM) and P3(M)) also show small range and agree well with the young birds.

The post-breeding grown feather (P9) from both populations shows a large continuum of values indicating a transition from a $C_3$ to $C_4$ photosynthetic pathway dominated habitat. This is particularly evident in the Portuguese population (P9(M)) where birds are suspected to be wholly migratory. Stable isotopes of carbon from atmospheric $CO_2$ are differently fractionated by plant photosynthetic pathways resulting in distinct isotopic differences between $C_3$ and $C_4$ plants (Hobson 1999). This means that habitats can be distinguished according to the relative abundance of $C_3$ and $C_4$ pathway plants; $C_3$ pathway plants having relatively lower $\delta^{13}$C values (mean –27‰) compared with $C_4$ plants (mean -13‰) (Smith and Epstein 1971, Chamberlain *et al* 2000, Yohannes *et al* 2005). Geolocators suggest the lesser kestrel winters in habitat dominated by $C_4$ savannah, tropical grassland and shrub (Catry *et al* 2011a). Consequently, the P9 samples may contain both sub-Saharan migrants, whose values are strongly different (less negative) to those of the breeding area, and resident birds whose isotopic ratios are likely to be very similar to the $C_3$ habitat Iberian signal of the chicks. In order to separate birds of unknown migratory status in to migrants and residents, a maximum limit for the breeding area was defined using the 95th percentile of the P3 and P4 feathers (-21.2‰). Individuals with post-breeding grown feather (P9) values less negative than this threshold can confidently be considered as migrant (42.0% for the partially migratory population, mean ±SE $\delta^{13}CP9_f$ = -16.0‰ ±0.4, 82.1% for the migratory population, mean ±SE $\delta^{13}CP9_f$ = -18.2‰ ±0.3). Carbon values determined in this study to be indicative of sub-Saharan Africa are within the range of mean values found by other sub-Saharan African studies (eg Neto *et al* (2002) mean = -16.4‰, Reichelin *et al* (2010) mean = -18.3‰) including the black kite *Milvus migrans*, a raptor species with the same wintering distribution and diet as the lesser kestrel (Cortes-Avizanda *et al* 2011, mean -14.2‰). For birds with $\delta^{13}C_f$ P9 below the limit expected for the breeding area, determining its migratory status is not so straight forward.

Unexpectedly, the $\delta^{13}C$ of the P9 feather ratios from 3 of the 6 adults known from geolocators to have wintered in the Sahel, Africa, were below the breeding area threshold, and another was borderline (Figure 6.1a). This indicates flexibility in the timing of moult which means an unknown proportion of individuals whose P9 feather is isotopically similar to Iberia may be migrants who completed moult in Iberia, rather than resident birds. Carbon can therefore only successfully assign migratory status to birds who complete moult outside of the Iberian breeding area.

### 2) Deuterium values probably not correlated with the isotopic signature of local precipitation

If hydrogen isotopic ratios of feathers were predominantly influenced by the isotopic composition of local precipitation, then feathers grown in the breeding area should be isotopically similar to the chicks and the 95th percentile breeding area limits defined for each population. Instead, deuterium values of feathers from (mostly) adult birds grown in the breeding season (P4(PM) and P3(M)) were significantly higher compared to the juvenile males and chicks (Figure 6.1b), a mismatch that has been reported in studies of other raptor species (Meehan *et al* 2003, Smith and Dufty 2005 and Greenwood and Dawson 2011). Hobson *et al* (2009) noted in the American kestrel, a species closely related to the lesser kestrel, that individuals with feather deuterium more enriched than -20.0‰ departed strongly from the expected linear relationship with precipitation. Furthermore, other studies have suggested that in birds with low drinking water requirements, such as the lesser kestrel, drinking water only contributes between 18 ±3% (Wolf *et al* 2011) and 20–32% (Hobson *et al* 1999) to the hydrogen values of tissues, including feathers. In Japanese quail *Coturnix* *japonica*, a species with high water intake needs, Wolf *et al* (2013) found only a weak correlation between feather hydrogen and drinking water.

Patterns of deuterium enrichment across the moult were further investigated by analysis of every primary feather from 9 individuals found dead (Figure 2). Three birds, including a known juvenile female, showed low variance in any isotope and similarity to the Iberian threshold in all feathers (depicted in yellow and purple, the juvenile female as open diamonds, Figure 6.2). The remaining 6 individuals showed variance in one or more isotopes. The breeding season grown feathers (P4-7) of these 6 individuals were all consistently enriched above the 95th percentile threshold limit of the chicks and juveniles, whilst winter grown feathers tended to show less enrichment (Figure 6.2 c and d). According to the carbon isotopic ratios, 2 of the individuals showing isotopic variance (a male and a female) are long distance migrants who completed moult in Africa. This is because carbon values of feathers grown post breeding are all significantly less negative than values expected for Iberia (Figure 6.2b). However, in terms of deuterium, these birds are indistinguishable from other enriched birds (Figure 6.2d), which again suggests that hydrogen is not able to inform migratory status.

### 3) Nitrogen is not useful in identifying migratory status

There was no significant difference in nitrogen between breeding (P4(PM) and P3(M) and post-breeding (P9) grown feathers in either population. This suggests there is no shift in dietary trophic level between Iberia and Africa, which is consistent with a year round diet known to mainly consist of orthoptera (Catry *et al* 2011a, Limiñana *et al* 2012) and small rodents. The Spanish population was generally more enriched in nitrogen than the Portuguese population, particularly during breeding. This may be due to a higher diversity of foods at various trophic levels in the Spanish population. Spanish birds had a larger range of nitrogen in the winter grown feather than the Portuguese birds, presumably because migrant birds winter in less intensively farmed areas. However, the difference was not sufficient to identify migrants and nitrogen was therefore not useful for differentiating migrant from resident birds in either population.

### 6.5.3. Isotopes may be able to distinguish non-breeding (juvenile) females from adults.

In total 7 individuals (one male, 6 females) showed low isotopic variability in carbon (Figure 6.2a and b). One female was known to be a juvenile and has isotopic ratios consistent with Iberia throughout all feathers. Three birds (one female and 2 males) were known to be adults and may be either a) migrants that completed moult in Iberia, or b) resident birds. All confirmed adults with low $\delta^{13}C_f$ P9 values are in one of these 2 categories. However, whilst adult males can be identified from plumage characteristics, adult and juvenile females cannot reliably be separated by plumage. The age of a few females in each population was known from ring data (10 from the migrant population, 14 from the partial migrants). For the majority of females, although all birds were sampled in breeding colonies, their age status (adult or juvenile) during feather growth the previous year was not certain. Consequently, in addition to being categorised as migrant or resident, females may also be juveniles with no migratory status. Carbon is unable to distinguish between them. However, in Figure 6.2, all 3 birds known to be adult show consistently enriched deuterium in all breeding season grown feathers, whereas the known juvenile shows no enrichment. The lack of juvenile enrichment is consistent with the juveniles and chicks sampled in Figure 6.1. According to the main explanatory hypothesis for enrichment, deuterium may be useful in separating adults from juveniles.

### 6.5.4. Explanatory mechanisms for deuterium enrichment

Hydrogen incorporation in raptors species appears to be more complex in comparison to other avian groups, resulting in high levels of deuterium enrichment. Meehan *et al* (2003) and Greenwood and Dawson (2011) discuss 3 non-exclusive hypotheses that may explain the high levels of deuterium enrichment.

In the first hypothesis the consumption of migrant birds with a non-local isotopic signal may result in the expression of more enriched deuterium values. However, this is not relevant to the lesser kestrel whose diet year round consists mainly of orthoptera and small rodents, and shall not be considered further.

Secondly, the synthesis of feather keratin from proteins mobilized from stores laid down in the non-breeding season may lead to a disconnection from the local hydrogen signal at the time of feather growth. Understanding of source to tissue discrimination factors (enrichment or depletion) and the speed in which a dietary change is reflected isotopically in body tissues (turnover time) is better understood in carbon compared to hydrogen (Martínez del Rio *et al* 2009, Hahn *et al* 2012). Feathers are synthesised from amino acids transported by blood. By switching individuals from one isotopically distinct diet to another, studies have confirmed that carbon in whole blood and feathers update at similar rates (Bearhop *et al* 2002) and reflect diet over a short time period varying from 15.7 ± 2.1days in great skuas *Stercorarius skua* (Bearhop *et al* 2002) to 11.2 ± 0.8 days in dunlin *Calidris alpina* (Evans Ogden *et al* 2004) and 3.9 to 6.1 days in yellow-rumped warblers *Setophaga coronata* (Pearson *et al* 2003). However, it has also been demonstrated that feathers can be synthesised from carbon and nitrogen derived endogenously from protein stores (Fox *et al* 2009). Discrimination factors and turnover times are significantly more complex in hydrogen, and less well understood. Whereas carbon is derived entirely from diet, hydrogen can be derived from multiple sources including from prey, drinking water and metabolically created water (Hobson *et al* 1999, Wolf *et al* 2011, Wolf *et al* 2012, Wolf *et al* 2013), each source with its own dynamics and unquantified contribution to the overall signal. Any delay in hydrogen turnover would be very difficult to assess.

Investigations into the deuterium delay hypothesis by studying the relationship between deuterium values in blood plasma and feathers in American kestrels *Falco sparverius* were inconclusive (Greenwood and Dawson 2011). However, in woodrats, body water turnover had a half-life of 3-6 days in response to water intake (Podlesak *et al* 2008), in rock dove *Columba livia* isotopic equilibrium with drinking water was reached in 3.97 ±0.72 days at room temperature (McKechnie *et al* 2004) and in Japanese quail, Wolf *et al* (2012) found the turn over time of hydrogen in blood plasma was generally faster than for carbon -although this was not statistically significant due to small sample size and large differences between individuals. These studies suggest that hydrogen likely updates at a similar rate to carbon and therefore the signal laid down in feathers during synthesis should represent a relatively immediate timescale. Furthermore, deuterium ratios observed in this and other studies are significantly enriched compared to values expected for local precipitation in either the breeding or wintering zones, suggesting significant disconnect with local precipitation, not simply a delay in signal expression.

In the third hypothesis, the lighter isotopologues (those with lighter hydrogen and oxygen isotopes) are preferentially lost as a result of increased evaporative cooling due to heat stress. This may lead to pronounced enrichment of heavier isotopologues in body water and therefore of feathers. Greenwood and Dawson (2011) favour, though cannot confirm, this hypothesis as the dominant mechanism in their analysis of deuterium from American kestrels, a species closely related to the lesser kestrel. Similarly, Smith and Dufty (2005) and Powell and Hobson (2006) also feel that heat stress during feather moult is the most likely explanation. Data analyses were conducted in this study to explore this hypothesis in more detail.

### 6.5.5. Assessing the effects of evaporative cooling on feather deuterium

According to this mechanism, increased rates of evaporative cooling are experienced as a result of breeding effort and temperature regulation in hot weather and this significantly enriches body water isotopic composition in adults compared to chicks. The correlation between enrichment and body size arises because metabolism and heat loss are proportional to mass and surface area. Larger individuals have higher metabolism and energy requirements and generate more heat but have lower capacity for passive heat dissipation (due to a smaller surface area in relation to mass) and as a result have increased requirement for evaporative heat loss. As a result, the relationship between the isotopic signatures of feathers and precipitation breaks down making hydrogen unreliable as a predictor of migratory status. Unfortunately, in our study there was insufficient availability of breeding metrics data (eg fecundity, chicks hatched, chicks fledged) from the previous year when feathers were grown, to assess direct evidence for breeding stress on adults. However, the influence of heat stress and body size on deuterium enrichment could be measured in chicks.

## 1) Heat stress and deuterium enrichment in chicks

The impact of heat stress in wild populations is usually difficult to measure, however this study benefitted from the fact that the majority of lesser kestrels in Portugal nest in artificial nest boxes in which they experience very different temperature regimes depending on box aspect and construction material (Catry *et al* 2011b). Although our sample size was small, there was evidence for a positive correlation between temperatures over $30^0$C and deuterium enrichment (Figure 6.3a). The impact of body size (wing length) on deuterium was almost significant (Figure 6.3b) and, based on results of other studies of enrichment in nestlings (eg Betini *et al* 2009), these results would be significant with a larger sample size. Although this study could not distinguish the relative effects of body size (chicks age) and temperature on enrichment, the difference in enrichment between the least and most enriched chicks (17.4‰) was similar to the value derived in heat stressed doves (20.2‰, McKechnie *et al* 2004).

## 2) Breeding and heat stress in adults

In support of the breeding stress hypothesis, Greenwood and Dawson (2011) found enrichment of adults to be correlated with body size (larger males were more enriched) and breeding effort (adult females that fledged higher mass female chicks had greater enrichment). In this study we speculated that the breeding season feathers of the migratory Portuguese population (P3M) were more enriched in deuterium than the Spanish population (P4PM) because an unknown proportion of adults likely bred in hot nest boxes and therefore likely experienced a higher level of heat stress. However, despite size dimorphism in the lesser kestrel, there was no effect of sex on enrichment of the P4(PM) feather grown at the start of the breeding season, or on the P3(M) grown at the end of the breeding season. There was also no effect of body size (wing chord length) on enrichment levels of either breeding season feather. This was attributed to high variation in degree of enrichment between individuals during the breeding season, particularly in the P3 feather, with some birds being considerably more enriched than others (Figure 6.1, Figure 6.2). In turn, this suggests various non-exclusive individual-specific effects (metabolism, individual fitness, stress levels and body condition). Effort related to breeding (fecundity, the number of chicks to hatch, number of chicks to fledge and partner quality) are likely also important factors.

Levels and reactivity of physiological stress hormones (eg corticosterone) in wild birds are known to vary seasonally and to be much higher in the breeding season than during post breeding, migration and wintering. Increased corticosterone, an adrenal steroid hormone, is linked with increased levels of activity and foraging in birds. For example, baseline levels of corticosterone were 50% lower in autumn compared to during breeding in long eared owls *Asio otus* (Romero *et al* 2009). Baseline levels are known to vary between Accipiter species and, in some species, between sexes. Cooper's hawk *Accipiter cooperii*, the subject of the study by Meehan et al (2003), mentioned above, is known to have relatively high baseline levels of adrenocortical (Rogers *et al* 2010), which may be a contributing factor in the extremely enriched deuterium observed by the authors. In American kestrels (the subject of Greenwood and Dawson's 2011 paper, also mentioned above) female corticosterone was significantly higher during the egg laying period (Rehder *et al* 1984). Most crucially for this study, in non-breeding females corticosterone levels were always lower and no significant peaks were observed (Rehder *et al* 1986). Flat corticosterone levels have also been observed in other wild, non-breeding avian species (Akesson and Raveling 1981) and differences in corticosterone may be an explanatory factor in varying reproductive success (Rehder *et* al 1986). This may explain the differences in deuterium enrichment observed between adult and suspected juvenile birds in this and other studies. It was also noted by Rehder *et al* (1986) that levels of plasma corticosterone rose post breeding in both breeding and non-breeding American kestrels. This was interpreted as preparation for migration, and has since been found in many avian species (Rogers *et al* 2010), and may also explain the upward trend in enrichment between the P1 and P10 feathers observed in all bar one of the 9 lesser kestrels in Figure 6.2 of this study.

Unlike most passerine species, which delay moult until after breeding and have good links between feather deuterium and precipitation, raptors undergo moult during the extremely stressful breeding season. This likely has two effects: firstly, to increases energetic demands further, and secondly the breeding season stress response becomes stored in feathers as a reliable summary of stress responses during feather growth (Bortolotti *et al* 2008, Bortolotti *et al* 2009) and likely, also manifests as deuterium enrichment. This phenomenon is not exclusive to raptors. In fact, deuterium enrichment of adult compared to juvenile feathers has also been noted in passerines that moult immediately after breeding (Powell and Hobson 2006, Marquiss *et al* 2012). It has also been suggested in corvids (Hobson *et al* 2004).

As levels of stress hormones such as corticosterone are individual responses and are not related to body size, this may explain why this study did not find a link between deuterium enrichment and wing length and why other studies found only tenuous links between breeding effort and deuterium enrichment (Greenwood and Dawson 2011). The corticosterone response in feathers of birds of breeding age may also explain why the linear relationship between deuterium composition and precipitation in juvenile feathers is unaffected, resulting in the successful generation of isoscapes with juvenile raptor feathers (eg Lott and Smith 2006) but why the link to precipitation breaks down in adult birds.

Many strands of evidence therefore suggest that breeding stress is the main explanatory mechanism for the level and pattern of deuterium enrichment observed in adult feathers of this and other raptor studies. Future studies should investigate this further by simultaneous assay of both corticosterone and hydrogen isotope ratios deposited in feathers of both breeding and non-breeding raptor and non-raptor species. Furthermore, recent advancements in tracking equipment and accelerometry should allow detailed derivation of ODBA (overall dynamic body acceleration), metabolism and energetics which could significantly increase understanding of the link between deuterium enrichment and stress.

### 6.5.6. Identification of non-breeding females using deuterium

According to the hypothesis of deuterium enrichment due to increased evaporative cooling as a response to breeding stress, non-breeding birds should be identifiable by their consistently low, flat levels of deuterium enrichment. In comparison, breeding birds (therefore adults) should have highly enriched breeding season feathers. This is observed in Figure 6.4. Here all young birds are significantly lighter in deuterium than the majority of confirmed adults. The females are of unknown age because juvenile birds cannot be identified by plumage, and span a large range of values. This indicates both adults and juveniles are present in the sample.

Birds with low enrichment can be identified by applying the base-line deuterium enrichment threshold derived for each population from the 95th percentile of the young birds. Consequently 6.0% of females of unknown age from the migratory breeding colonies and 5.3% from the partially migratory colonies were identified as non-breeding. It is not certain whether these individuals are juveniles or non-breeding adults. In addition, Figure 6.4 shows that 7.9% of confirmed adults from the migratory population (Portuguese P3(P)) and 1.6% from the partially migrant (Spanish P4(S)) population also have extremely low enrichment of breeding season feathers, comparable to levels expected in young birds. Interestingly, one of these adults with low enrichment of the P3 feather was the male depicted in purple in Figure 6.2. This bird showed strong enrichment of early breeding season feathers (P4-7) then a sharp drop in the P3 feather value. Interpretation of this individual in accordance with the corticosterone/breeding stress hypothesis allow speculation that this bird had a breeding attempt but likely failed, hence the sudden drop in P3 enrichment in comparison to other birds. This suggests that feathers grown early in the breeding season (P4 and particularly P5) may be more reliable indicators of whether or not a breeding attempt was initiated. This may explain why fewer confirmed adults sampled for the early breeding season P4 feather were highlighted as possibly non-breeding birds. Consequently, the 5.3% of unenriched females of unknown age status from the partially migrant population, sampled for P4, are highly likely to be juveniles with flat levels of enrichment across all feathers and can be excluded from studies of migratory behaviour. The slightly higher percentage (7.9%) of females of unknown age from the Portuguese population sampled for the end of breeding P3 feather may include adults misidentified as juveniles.

### 6.5.7. Confirmation of juvenile status using Nitrogen

The range of expected values of nitrogen for both adults and chicks was very small (Figure 6.1). Despite this, the slight difference in the means of chicks and juvenile males in comparison to their respective P4(PM) or P3(M) feather was statistically significant. This suggests the diet of chicks differed from the adults, possibly indicating they are selectively provisioned with smaller or softer bodied prey items by the adults. The range of juvenile male nitrogen values overlapped considerably with the P4(PM) which is likely because diet becomes less selective in older chicks who are able to handle a larger variety of prey. This range overlap meant nitrogen was not informative in confirming the age status of birds with low deuterium enrichment in the Spanish population. However, there was less overlap with young birds in the Portuguese population (Figure 6.1). After regrouping birds by their age status according to carbon and deuterium (Figure 6.5), all females of unknown age that were light in terms of both carbon and deuterium, had nitrogen values indistinguishable from chicks and different to the known adults. Thus, these individuals (6.0% of individuals in the Portuguese sample) are highly likely juvenile birds of no migratory status and can be eliminated from studies of migratory behaviour.

**Table 6.1** *Status of lesser kestrels determined by stable isotopes (%).*
*Birds of unknown migratory status are either resident or birds who completed moult in Iberia prior to migration. Percentages are presented both including and excluding birds determined by deuterium to be juveniles.*

| **Status** | **Migratory population (Portugal)** | | **Partially migrant population (Spain)** | |
|---|---|---|---|---|
| | **Including juveniles (%)** | **Excluding juveniles (%)** | **Including juveniles (%)** | **Excluding juveniles (%)** |
| **Migrant** | 82.1 | 87.3 | 42.0 | 44.4 |
| **Migratory status unknown** | 11.9 | 12.7 | 52.7 | 55.6 |
| **Juvenile** | 6.0 | | 5.3 | |

Although the final sample size of juvenile females was small, nitrogen supports the theory that deuterium enrichment due to increased evaporative cooling resulting from breeding stress can be used to identify non-breeding (juvenile) individuals within a population.

### 6.5.8. Individual migratory status in migratory and non-migratory populations

A summary of individual migratory status derived for the 2 populations by this study is presented in Table 6.1. After exclusion of juveniles, the majority (87.3%) of individuals from the Portuguese population, suspected to be wholly migratory, were successfully assigned as migratory by carbon. In this population, it is likely that all the birds with unknown migratory status (12.7%) were birds that moulted in Iberia prior to migration. It is known from tracking studies that birds perform a short post breeding period of local wandering prior to migration (Catry *et al* 2011a) and moult could have been completed during this phase. As expected, the proportion of birds assigned by carbon as migratory was significantly lower in the partially migrant population (44.4%). Assuming the proportion of birds to complete moult in Iberia is approximately the same as in the migratory colony, potentially 42.9% of birds were resident. Unfortunately, it is not possible to determine isotopically which individuals are true residents.

## 6.6. Conclusions

Of the 3 isotopes tested only carbon was useful when assigning individual migratory status in the lesser kestrel. However, isotopic ratios of individuals fitted with geolocators indicated flexibility in timing of moult. This means an unknown number of migratory individuals may have completed moult in Iberia rather than being truly resident. Carbon could only robustly identify migrants meaning this isotope was less informative in determining migratory status in the partially migrant population. This emphasises that understanding the moult characteristics of the study species, in terms of moult flexibility as well as order of feather moult, is crucial to accurate interpretation.

This study provides additional support for a breeding stress hypothesis as the dominant explanatory mechanism for enriched deuterium seen in many raptor species. If increased rates of stress are experienced as a result of breeding effort and temperature regulation in hot weather, then adult breeding season feathers should consistently be significantly enriched in deuterium in comparison to juveniles and non-breeding birds. Evidence for this was found in this study and verification of juvenile status was independently provided by nitrogen. This allowed elimination of juvenile females, which are indistinguishable from adults by plumage. This new interpretation of deuterium in raptors has many potential applications across a wide range of species and research questions.

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# Chapter 7

# General Conclusions

**Photo:** *Left: Stork with leg ring code 4C+ equipped and ready for release.*

*Right: Tagged stork 4P+ with chicks.*

## 7.1. Key findings

### 7.1.1. White storks, insights for conservation management planning

This work is a contribution to our understanding of the movement and foraging ecology of a newly resident species in response to an abundant, artificial food supply. In a changing world where many species including bears, gulls, garden birds and marine species (Ayres *et al* 1983, Orams 2002, Yoril and Caille 2004, Robb *et al* 2008a, Fuller *et al* 2008, Corcoran *et al* 2013) are adapting their wintering and breeding ecology around anthropogenic food supplements, this knowledge is hugely relevant.

Climate and environmental changes in the form of milder winters and abundant food resources (landfill and red swamp crayfish) have enabled the white stork to remain on the nest as a central place forager throughout the year. This is notably rare in temperate climes, where species are normally highly mobile during winter as a response to limited food resources (Newton 1998). This fascinating new behaviour in resident storks governs many aspects of their wintering and breeding season foraging strategies. Thus, the nest-landfill distance determined the frequency of landfill use, foraging range in non-landfill habitat and proportion of GPS fixes spent foraging (Chapter 2). It was also discovered that, for the years of study, the nest - landfill distance was more important than climatic variables in predicting landfill use (Chapter 3). This may indicate that, for this species, the climatic drivers of winter habitat use are currently being obscured by the anthropogenic contribution to the abundance of winter food.

From a conservation perspective, this thesis provides key insights into how the artificially high white stork population in Iberia may be affected by the imminent closure of open-air landfills. From their movement behaviour and habitat, it was ascertained that birds travelled to visit landfill from further away than previously estimated (Tortosa *et al* 2002, Massemin-Challet *et al* 2006). This indicated that the sphere of influence of landfills may be greater than previously suspected, therefore a higher proportion of the population than previously thought may be regularly utilizing landfill resources and will consequently be impacted by their closure. This study also provided the first quantitative evidence of the importance of landfill for breeding success (Chapter 4). High productivity near landfills indicates the important role this food resource had been in the strong population recovery observed in the last few decades. Frequency of visits to landfill declined with increasing nest-landfill distance, never the less, it can be speculated that even rare landfill visits may be the crucial difference between nest success rather than failure, particularly in years with low food resource availability in local habitats. However, reproductive rates away from landfills were low compared to other studies across the breeding range (Barbraud *et al* 1999, Tryjanowski *et al* 2004, Hilgartner *et al* 2014) so it is not yet known whether population dynamics in Iberia will remain stable.

A caveat of this study is that nests far from landfill were considered to be minimally influenced by them. However, it is highly likely that the majority of storks (resident and migrant) benefited from landfill use to some extent during winter (Peris 2003), thereby improving individual fitness. Carry-over effects, whereby events of one season influence the success of an individual in another, are thought to be widespread (Harrison *et al* 2011), and evidence indicates that diet quality during winter can influence breeding success the following spring (Gill *et al* 2001, Sorensen *et al* 2009, Robb *et al* 2008b). Thus, productivity in colonies that do not use landfill during breeding are still boosted by landfill use during non-breeding and are therefore higher than they otherwise should be. The implications of this are that true non-landfill productivity rates in Iberia will actually be lower after landfill closure than predicted by this study.

The combination of landfill closures and rapid climatic change predicted for Iberia makes the white stork particularly susceptible to future environmental change. Increased utilization of rice field habitat after landfill closure may bring storks into increased conflict with farmers who dislike large flocks of storks because they muddy the water in rice paddies and trample young crops. Management alternatives, such as the creation of artificial feeding sites, would mitigate the impact of landfill closure for white storks and other species that use landfills, including herons, egrets, gulls, and raptors.

### 7.1.2. Stable isotopes as indicators of migratory status: the importance of moult

In a changing world, where increasing numbers of species are becoming sedentary where they were formerly wholly migratory, it will be increasingly of interest to be able to separate residents from migrants within the same population. This study emphasises that full understanding of moult is crucial to accurate interpretation of stable isotope data, both in terms of the sequence and timing of feather renewal. It was surprising that four of the birds tagged with geolocators had carbon ratios similar to Iberia when they were known to have wintered in Africa (Chapter 6). As a result, it was not possible to separate true residents from migrants that completed moult in Iberia. Variance in moult between individuals is often not well understood, so caution is urged.

My work contributes to mounting evidence that timing of moult may also be influencing hydrogen incorporation in feathers. Whilst many studies find hydrogen is linked to local precipitation (Hobson *et al* 2012) others find ratios of deuterium in feathers from the same location are different between age classes and enriched beyond values predicted by precipitation (Meehan *et al* 2003, Smith and Dufty 2005, Lott *et al* 2006, Powell and Hobson 2006, Hobson *et al* 2009, Greenwood and Dawson 2011). The mechanisms driving this detachment from meteoric water are still unclear but theories suggest that simultaneously moulting whilst breeding may be recording breeding season processes in feathers, particularly extra evaporative cooling due to breeding effort. This thesis ventures a new hypothesis for further consideration: that levels of stress hormones during breeding (breeding stress) may be influencing feather deuterium.

## 7.2. Future research directions

### 7.2.1. White storks

This thesis provides a baseline for the current situation with white stork movement behaviour and productivity in relation to landfill use. Sadly, the period of my doctoral research ended just as the landfill closures were finally coming into effect –in other words, just as things got really interesting!

The planned closure of open-air landfills is a fantastic and rare opportunity for studying the consequences of an abrupt change in food supply on movement behaviour and habitat selection, population dynamics and productivity. Furthermore, the white stork is a prominent and well-studied species which enables very in-depth research questions to be tackled. This will facilitate greater understanding of species response to anthropogenic environmental change.

In order to understand the responses to these changes, individuals need to be followed. Consequently, GPS tags should continue to be deployed on white storks in colonies close to landfills in order to monitor the consequences of closure for movement behaviour and productivity and also for migratory decisions.

My research highlighted that current levels of white stork productivity on the Iberian Peninsula are low compared to breeding populations elsewhere in Europe, particularly in colonies far from landfills. Future research should investigate whether reproductive rates are sufficient to sustain a stable population and, if necessary, devise management strategies.  I have started to address this question using survival analysis of 20 years of white stork chick ringing data from Portugal, coupled with historic productivity data and population estimates from the recently conducted 7th International White Stork Census (2014). However, the manuscript is still a work in progress so was not included in this thesis.

### 7.2.2. Lesser Kestrels

In this thesis, interpretation of deuterium enrichment was carried out with low sample sizes. This was because understanding the processes and implications of enrichment was not part of the original study design. Feather sampling targeted adult birds because of interest in migratory behaviour, so only a limited number of individuals transpired to have juvenile feathers. Future research should focus on obtaining good sample sizes of both breeding and non-breeding birds, with the breeding history corresponding to the year of feather growth. Levels of stress hormones, such as corticosterone, in feathers should then be assayed simultaneously with deuterium in order to compare breeding and non-breeding individuals. This would facilitate fuller understanding of the impact and variability of stress levels in individuals on deuterium ratios. This may have profound implications for the interpretation of stable isotopes of hydrogen from feathers across all species.

I have a considerable amount of data that has not been included in this thesis. In the course of my research I analysed stable isotopes of carbon, nitrogen and hydrogen from 12 passerine species resident in the African Sahel and a further 6 resident species from Southern Africa. This was part of my attempt to understand variability in deuterium derived from lesser kestrels. Additionally, I performed triple-isotope analysis on feathers from the European Roller *Coracias garrulous* with the aim of compiling a multi-species analysis of variability in feather isotopes between the Iberian breeding area and African (Sahel and Southern Africa) wintering areas. The aim remains to bring these data to publication.

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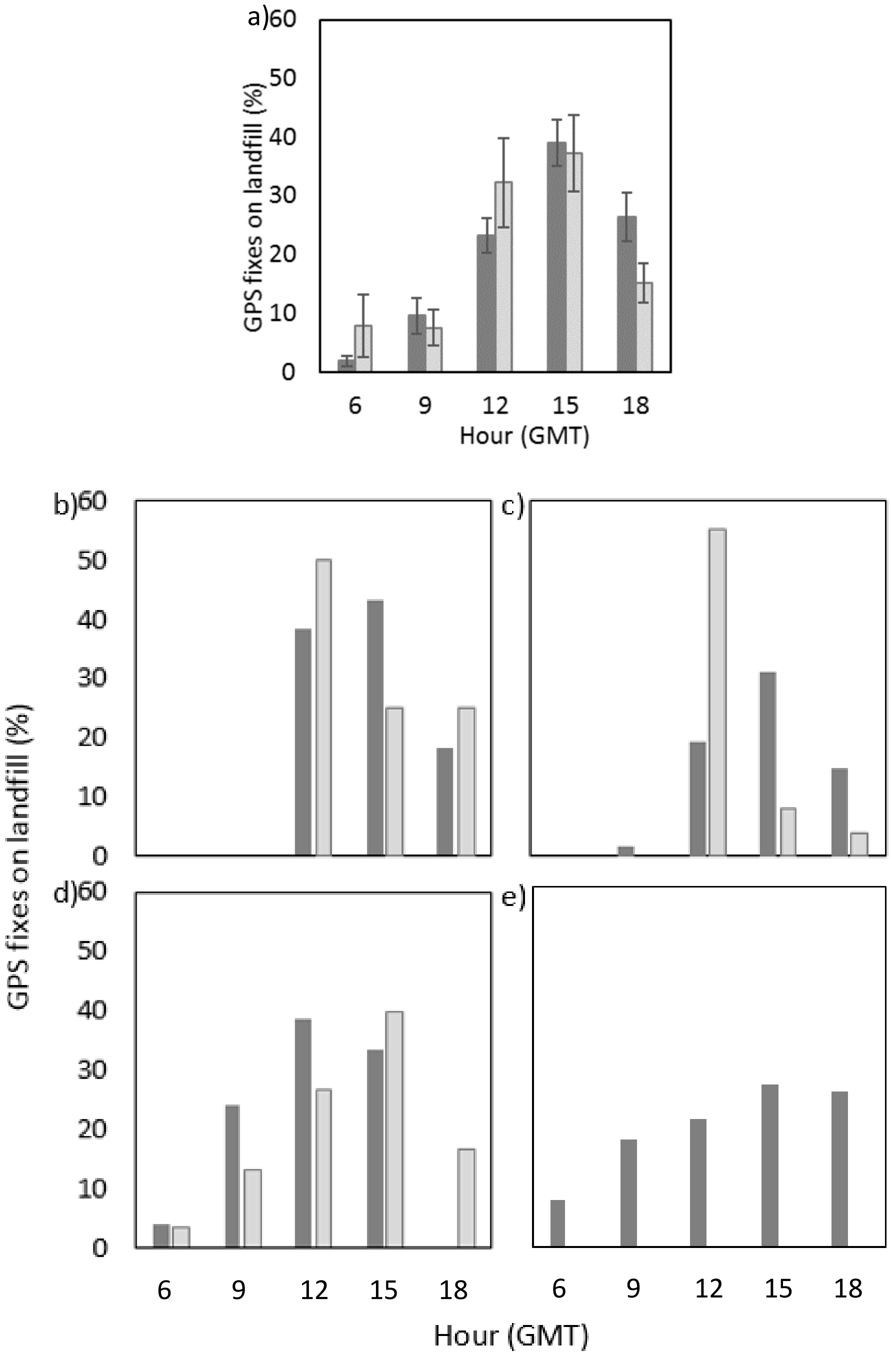
# Appendices

## Appendix 2.1

Confusion matrix. Performance of the decision tree model used to predict four behaviour classes: 1) standing/preening, 2) foraging, 3) flight and 4) tending eggs. True positives are in bold.

| | | Behaviours | | | |
|---|---|---|---|---|---|
| | | Standing / Preening | Foraging | Flight | Tending Eggs |
| Predicted | 1 | **1205** | 41 | 5 | 16 |
| | 2 | 23 | **749** | 5 | 0 |
| | 3 | 0 | 1 | **82** | 0 |
| | 4 | 16 | 0 | 0 | **107** |

**Appendix 2.2** Temporal patterns of landfill attendance



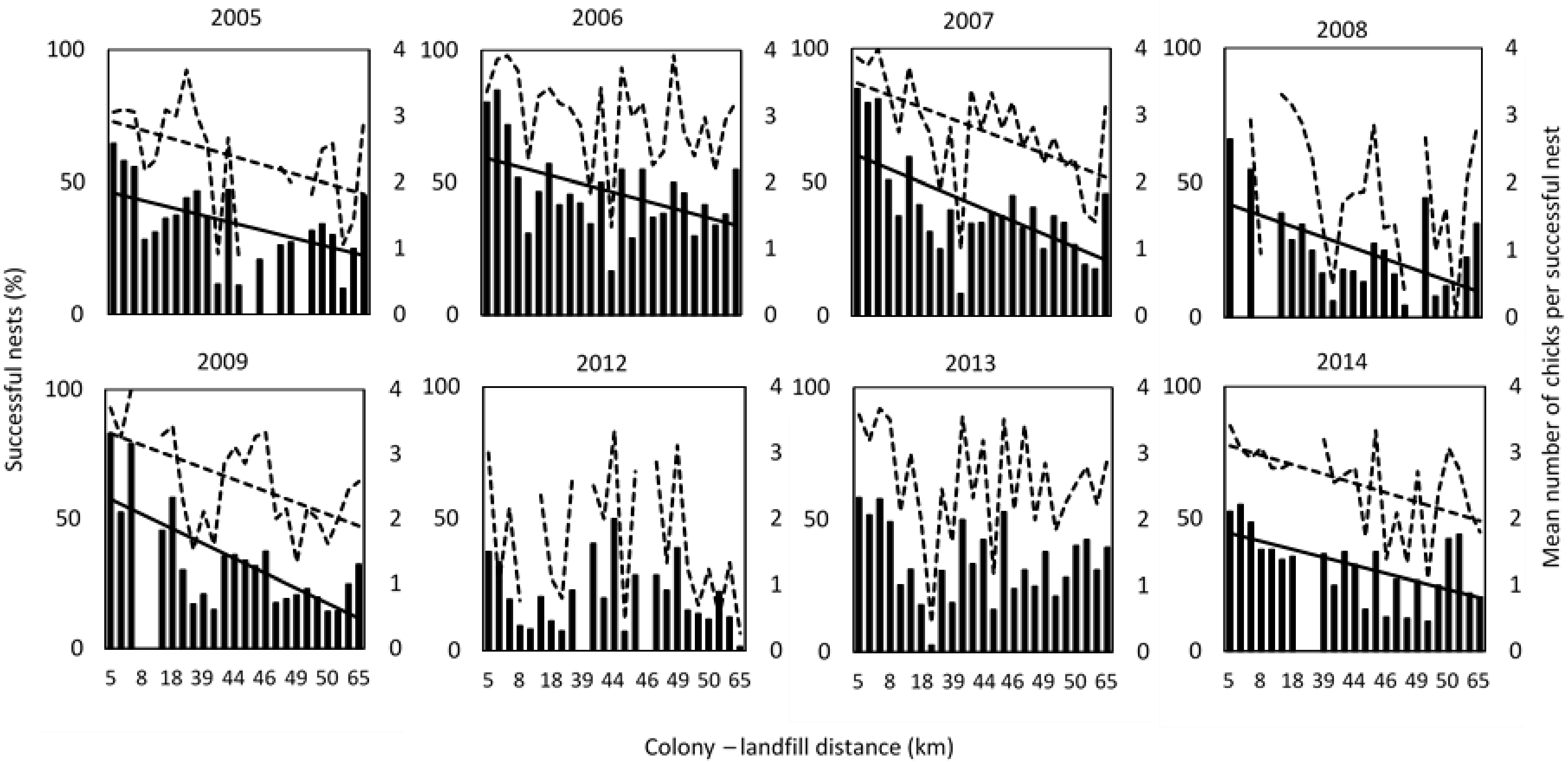
Distribution of GPS fixes occurring on landfill during each of the 5 daily data bursts as a percentage of all transmissions on landfill during the non-breeding (dark grey) and breeding (light grey) seasons. Graph a: Summary (± SE) of 10 birds tracked in 2012-13. Graphs b-e: variability in landfill attendance by individuals nesting at different distances from landfill b) 3.5 km, c) 4.1 km, d) 14.2 km, e) 25.0 km. The latter individual only used landfill during the non-breeding season. In all graphs, dark bars are the non-breeding season, light bars are the breeding season.

**Appendix 3.1**

Outputs of the GLMM investigating predictors of winter nest use.

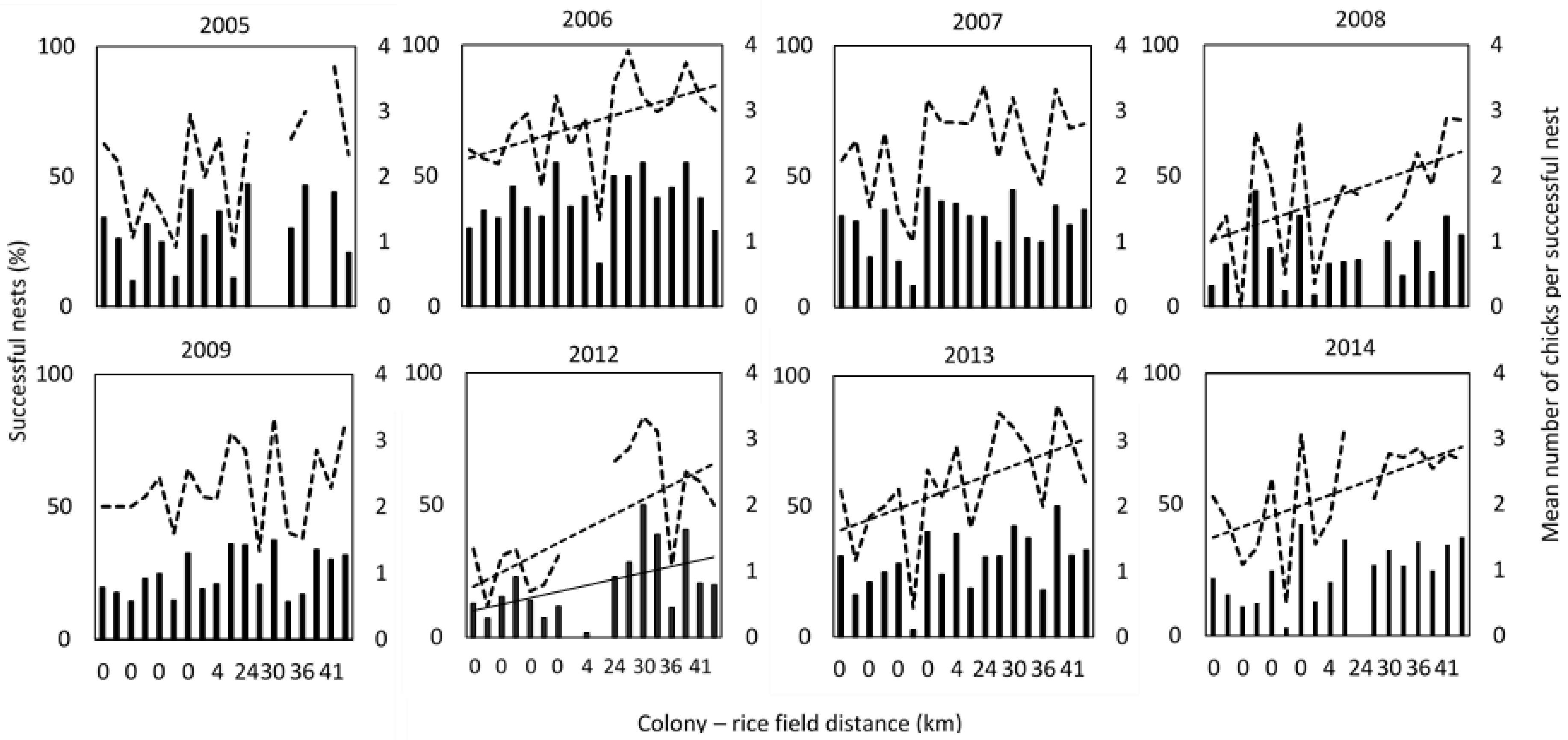
| **Predictors** | **Estimate** | **SE** | **Z** | **Pr(>\|z\|)** |
|---|---|---|---|---|
| Intercept | -0.758 | 0.096 | -7.932 | < 0.001 |
| Maximum temperature ($^0$C) | -0.014 | 0.025 | -0.547 | 0.585 |
| Minimum temperature ($^0$C) | -0.010 | 0.026 | -0.766 | 0.444 |
| Wind speed (m/sec) | 0.008 | 0.026 | 0.302 | 0.762 |
| Nest-landfill distance (km) | -0.05 | 0.062 | -0.860 | 0.390 |
| Daily Precipitation (mm) | -0.040 | 0.026 | -1.541 | 0.123 |
| Mean precipitation 10 (mm) | 0.025 | 0.024 | 1.058 | 0.290 |
| | | | | |
| Interactions | | | | |
| Daily Ppt – Wind speed | -0.037 | 0.019 | -1.880 | 0.062 |
| Daily Ppt- Nest-landfill dist | -0.015 | 0.021 | -0.733 | 0.464 |
| Daily Ppt – Ppt 10 | | | | |
| Daily Ppt – Max temp | 0.006 | 0.030 | 0.188 | 0.851 |
| Daily Ppt – Min temp | 0.011 | 0.025 | 0.427 | 0.669 |
| Nest landfill dist – Max temp | 0.004 | 0.024 | 0.177 | 0.859 |
| Nest landfill dist – Min temp | -0.023 | 0.024 | -0.973 | 0.331 |
| Nest landfill dist – Wind speed | -0.040 | 0.026 | -1.562 | 0.547 |
| Nest landfill dist – Ppt 10 | -0.023 | 0.022 | -1.058 | 0.290 |
| Ppt 10 – Max temp | -0.009 | 0.024 | -0.401 | 0.688 |
| Ppt 10 - Min temp | -0.006 | 0.019 | -0.292 | 0.770 |
| Ppt 10 – Wind speed | 0.021363 | 0.024 | 0.881 | 0.379 |
| Max temp – Wind Speed | 0.03587 | 0.023 | 1.555 | 0.120 |
| Max temp – Min temp | 0.009984 | 0.023 | 0.428 | 0.668 |
| | | | | |
| Random effects | Variance | SD | | |
| Individual | 0.053 | 0.230 | | |
| Landfill used | 0.002 | 0.044 | | |
| Year | 0.011 | 0.107 | | |

**Appendix 4.1a:** Colony-scale productivity in relation to the distance between the centre of the colony and nearest landfill, years 2005 – 2009 and 2012 - 2014.



Dashed lines are the percentage of monitored nests in each colony that successfully reared at least one chick. Bars indicate the mean number of chicks per nest (including failed breeding attempts) per colony. Dashed trend lines indicate years where the percentage of successful nests was significantly related to the colony – landfill distance and the bold trend line indicates where the mean number of chicks per nest was also significant.

**Appendix 4.1b:** Colony-scale productivity in relation to the distance between the centre of the colony and nearest rice field, years 2005 – 2009 and 2012 - 2014.



Dashed lines are percentage of monitored nests per colony that successfully reared at least one chick. Bars show mean number of chicks per nest (including failed breeding attempts) per colony. Dashed trend lines indicate years where the percentage of successful nests was significantly related to colony – rice field distance, bold trend lines indicate where the mean number of chicks per nest was also significant. Only colonies ≥25km from landfill sites are included to avoid observing impacts of landfill on productivity.

**Appendix 6.1**

Carbon isotopic ratios of Portuguese post-breeding grown feather (P9(M) sampled in 2009 and 2013.

There is an inter-annual difference in carbon means, but not in the range of carbon values.