

Greater flamingos *Phoenicopterus roseus* are partial capital breeders

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Capital breeding refers to a strategy in which birds use body stores for egg formation, whereas income breeders obtain all resources for egg formation at breeding sites. Capital breeding should occur more in large-bodied species because the relative cost of carrying stores for egg formation becomes smaller with increasing body size. Based on a comparison between stable isotopes of carbon and nitrogen in potential prey at wintering sites and eggs, we examined whether greater flamingos use nutrients stored earlier in the year for egg production. Our results suggest that the greater flamingo is a partial income breeder, since prey for egg formation were obtained both in overwintering sites and at the breeding site. This may be because there are selective pressures for nesting females to lay soon after arriving at the breeding site, which may be facilitated by arriving at the breeding site with developed ovarian follicles.

Many bird species accumulate body stores from feeding at stopover sites when moving from wintering to breeding sites. These body stores may be used later in egg formation, a strategy known as capital breeding (Drent and Daan 1980, Jönsson 1997, Meijer and Drent 1999). Other species rely on resources acquired at breeding sites for egg production, a strategy termed income breeding (Drent and Daan 1980, Jönsson 1997, Meijer and Drent 1999). It is assumed that the capital breeding strategy has evolved in species breeding in sites with low food availability at the time of laying, such as in the Arctic or in unpredictable habitats, and also that it should be more prevalent in large-bodied species, because it may be energetically costly for small birds to carry sufficient body stores for egg formation from stopover sites along migration routes to breeding sites (Klaasen et al. 2001, Klaassen 2003). Capital and income breeding may, in fact, be the extremes of a continuum, and at least some large-bodied arctic-nesting species may be partial income breeders (Gauthier et al. 2003, Schmutz et al. 2006).

The greater flamingo *Phoenicopterus roseus* is the largest waterbird of western Mediterranean wetlands. Greater flamingos move from southern Spanish wintering quarters to breeding sites between one month and a week before breeding (Rendón-Martos et al. 2009). Strong competition between individuals for nest sites (Rendón et al. 2001) may select for nesting as soon as possible after arrival at breeding sites. It would therefore be adaptive for greater flamingo females to accumulate resources for egg formation at wintering sites, which seems plausible because of the

occurrence of many females with distended abdomens at such sites in late winter (authors' unpubl.).

In this study, we used carbon and nitrogen stable isotopes in potential prey and eggs of flamingos to determine the degree to which resources acquired from overwintering localities around (B 250 km) a breeding site were allocated to egg nutrients.

Methods

The greater flamingo is a protected species in Spain, and it is not possible to enter colonies without provoking colony desertion by incubating adults. In early May 2006, a partial colony desertion event was induced by red foxes *Vulpes vulpes* at Fuente de Piedra lake, Spain (37° 06'N, 48° 45'W), and this gave us the opportunity to collect 15 newly laid (i.e., clean, white) greater flamingo eggs. Eggs were kept in a refrigerator (4°C) and transported to a laboratory a day after collection.

Egg yolk was separated by hand, and sub-samples obtained using a syringe. Yolk sub-samples were freeze-dried for 48 h and powdered (without lipid removal) using a mortar and pestle.

Greater flamingos are mainly filter feeding, ingesting particles in a size range of 500–6 000 µm gathered using rows of plates that line their beaks (Zweers et al. 1995), although some prey can be located by sight. Greater flamingos' diet is very wide, although they feed mostly on aquatic invertebrates (bivalve molluscs, crustaceans, and

insects) as well as their eggs and larvae (Johnson and Cézilly 2007). In April 2007 we collected potential prey of greater flamingos in seven wetlands 250 km from Fuente de Piedra lake: Salinas de Cabo de Gata, Salinas de Cerrillos, Salinas de la Tapa, Marismas del Odiel, Marismas del Guadalquivir, Salinas de Bonanza, and Veta la Palma fish farm, as well as Fuente de Piedra (Fig. 1). These are the most important wetlands for greater flamingos in southern Spain during overwintering (Amat et al. 2005, Rendón et al. 2008, Rendón-Martos et al. 2009). Sampling sites were located in the areas where flamingos fed. We take the samples from the water column and the bed sediments, using a nylon mesh hand net during at least 15 minutes per site. Prey samples mainly included brine shrimps (*Artemia* spp.), cladocerans (*Daphnia* spp.), insects (Diptera, Coleoptera, Heteroptera, Odonata), Decapoda (Palae-

monidae), and Isopoda. After collection, all prey were stored in ethanol, which does not modify stable-isotope signatures (Hobson et al. 1997). In the laboratory, prey were sorted to the lowest taxonomic category as possible, and then oven dried at 50°C for 48 h, and subsequently powdered using a mortar and pestle.

Analyses of stable isotopes of whole egg yolk and prey were conducted at the Iso-Analytical Ltd. Laboratory (Crewe, UK) using elemental analysis–isotope ratio mass spectrometry to obtain values for carbon (C) and nitrogen (N) concentrations, $^{13}\text{C}/^{12}\text{C}$ and $^{15}\text{N}/^{14}\text{N}$, both relative to reference material, and expressed in delta notation ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, respectively). With this procedure δ values were obtained for each sample, so that $\delta X (\text{‰}) = ([R_{\text{sample}}/R_{\text{reference}}] - 1) \times 1000$, where X denotes the heavier isotope and R the ratio of heavy to light isotope (e.g.,

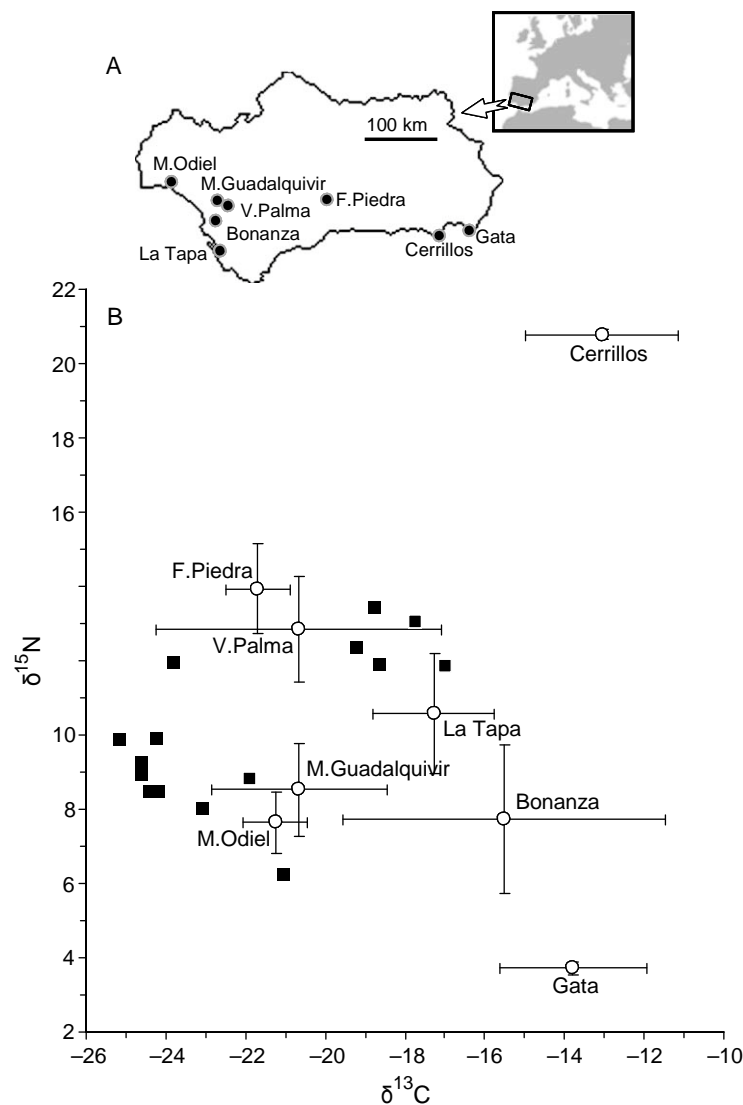


Figure 1. A Study area showing the location of the sampling localities in Southern Spain. B Individual (filled squares), mean \pm SD $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ in whole egg yolks of greater flamingos. Mean \pm SD stable C and N isotopes of potential prey in feeding areas (open circles) are also shown.

Gannes et al. 1998, Kelly 2000, Fry 2006). The reference material used during analyses was Iso-Analytical Working Reference Standard IA-R042 (powdered bovine liver) with a $d^{13}C$ value of -21.60‰ vs Vienna-Peedee Belemnite, and a $d^{15}N$ value of 7.65‰ vs air. Standard deviations of laboratory standard bovine liver run concurrently with samples of eggs and prey were estimated to vary between 90.05 and 90.07‰ for carbon, and between 90.09 and 90.13‰ for nitrogen.

We corrected for lipids in yolk with an arithmetic correction using published parameters, for which we used the equation: $d^{13}C_{\text{corrected}} = d^{13}C \mp 0.82 > D$ (see Oppel et al. 2010), where $d^{13}C$ is the value measured in the whole yolk sample, and D is the difference in $d^{13}C$ between yolk lipids and lipid-free yolk. We used the average D for carnivorous birds ($D=5\text{‰}$, Oppel et al. 2010). Despite Oppel et al. (2010) caution against the use of arithmetic lipid correction of egg yolk $d^{13}C$ for wild birds that obtain macronutrients from different ecosystems (e.g., freshwater vs saline), we used this method of correction because greater flamingo both overwinter and breed in saline wetlands (Amat et al. 2005). On the other hand, the large number of sources analysed and the large variability in isotopic ratios relative to distance between sources present in our data must have diminished the effects of lipid correction on diet estimates (Tarrow et al. 2010).

To estimate the relative importance with which prey from each locality contributed resources to our sample of greater flamingo eggs from Fuente de Piedra lake, all prey from each locality belonging to the same order were grouped, and average stable-isotope values calculated. This was justified because there were no significant differences between prey for combined isotopes (Table 1, MANOVA: Wilks' lambda=0.72, $F_{14,110}=1.41$, $p=0.159$), neither in $d^{15}N$ (Table 1; ANOVA: $F_{7,56}=1.05$, $p=0.407$), nor in $d^{13}C$ (ANOVA: $F_{7,56}=1.82$, $p=0.101$), but there were significant differences in isotopic signatures between sites (Table 1; MANOVA: Wilks' lambda=0.07, $F_{14,110}=22.47$, $p<0.001$; ANOVA $d^{15}N$: $F_{7,56}=55.41$, $p<0.001$; ANOVA $d^{13}C$: $F_{7,56}=7.00$, $p<0.001$). Using such average values, we then fitted a Bayesian mixing model to prey data (SIAR, Parnell 2008), for which the isotopic values of prey were adjusted by fractionation factors (e.g., Fry 2006). The latter were

obtained from the literature for diets of carnivorous ducks: 3.13 for $d^{15}N$ and 0.06 for $d^{13}C$ lipid-free (Hobson 1995).

Results

There was large variation in both $d^{13}C$ and $d^{15}N$ of aquatic invertebrates sampled in the most important wetlands for greater flamingos in southern Spain (Table 1). There were large interindividual differences in both $d^{13}C$ and $d^{15}N$ among flamingo eggs (Fig. 1). Estimates from the SIAR mixing model suggest that nutrients allocated to egg production were obtained in all localities in which invertebrates were sampled, although the main localities were Marismas del Odiel (mean contribution 95% CI: 20%, 2–38%), Marismas del Guadalquivir (18%, 0–35%), and Veta la Palma (16%, 0–32%). Prey from the breeding site, Fuente de Piedra (18%, 0–32%), also made an important contribution to egg formation. The proportional site contribution of each of the remaining wetlands (La Tapa, Bonanza, Gata, and Cerrillos) to egg formation was $<10\%$.

A visual examination of Fig. 1 show two clusters of individuals in the space defined by $d^{13}C$ and $d^{15}N$ values, suggesting that the analysed eggs come from, at least, two distinct wintering groups. However, a two groups SIAR model show that the proportional contribution of each locality to egg formation does not differ significantly between clusters (paired Wilcoxon sign-rank test: $z=2$, $p=0.844$).

Discussion

In this study we did not attempt to estimate the relative importance of endogenous vs. exogenous resources on egg formation in greater flamingos, but simply to know whether flamingos obtained resources for egg formation in overwintering sites. Our results suggest that the greater flamingo is a partial income breeder, since prey for egg formation were obtained both in overwintering sites and at the breeding site. Indeed, the SIAR model estimated about 18% of egg nutrients were obtained in Fuente de Piedra.

Table 1. Mean \pm SD (n, sample size) stable-carbon and nitrogen-isotopes values per potential invertebrate prey type and foraging wetlands of greater flamingos in southern Spain, used to predict isotopic signatures of whole egg yolk of flamingos.

Order	Prey			Wintering area				
	n	$d^{15}N$	$d^{13}C$	Locality	Habitat	n	$d^{15}N$	$d^{13}C$
Anostraca	7	11.695.70	-19.293.29	Salinas de Bonanza	Salt pans	7	7.791.99	-15.594.04
Cladocera	3	8.892.16	-22.393.41	Salinas de Cerrillos	Salt pans	3	20.890.13	-13.191.92
Decapoda	9	12.994.40	-18.593.39	Salinas de Cabo Gata	Salt pans	2	3.790.16	-13.891.83
Isopoda	2	11.890.53	-20.092.09	La Tapa	Salt pans	4	10.691.61	-17.391.54
Heteroptera	19	10.692.10	-20.593.65	Odiel Marshes	Salt pans	6	7.690.84	-21.390.82
Coleoptera	7	8.591.91	-20.594.28	Guadalquivir Marshes	Seasonal marshes	15	8.591.25	-20.792.20
Diptera	15	10.994.49	-18.593.36	Veta la Palma	Fish farm	23	12.891.43	-20.793.57
Gastropoda	2	9.291.79	-12.894.94	Fuente de Piedra	Seasonal lake	4	13.991.21	-21.790.80

The period of rapid follicle growth, during which yolk accumulates in the ovarian follicle, is not known for greater flamingos. Judging from the occurrence of more females than males with hanging abdomens in wintering sites, females may start developing ovarian follicles in such sites, weeks before breeding. Group displays of greater flamingos are observed up to four months before breeding (authors' unpubl. data), and during such displays individuals emit calls that may induce ovarian follicle growth, as in other bird species (Lehrman and Freidman 1969). Arriving at the breeding site with almost completely developed ovarian follicles may be advantageous for nesting females, as they may lay soon after arriving at the breeding site. Those females that delay breeding experience strong competition for nesting sites, and have to delay laying and breed in low quality sites (Rendón et al. 2001). Therefore, it may be that late laying females may rely more for egg formation on resources acquired at the breeding site than early laying females, since local feeding will contribute to falling nutrient contributions from stores over time (the nutrient reallocation hypothesis of Ryder (1970), but see Gauthier et al. (2003)). Gauthier et al. (2003) found a seasonal variation in the relative use of exogenous and endogenous stores for egg formation in geese.

Our results support the conclusion of Meijer and Drent (1999) that exclusive capital breeding may be very rare in birds, and that, as a large species, the greater flamingo has an intermediate laying strategy along the capital-income continuum.

Acknowledgements – We were supported by grant CGL2005–01136BOS from Ministerio de Educación y Ciencia, Spain, with EU-ERDF support. Consejería de Medio Ambiente, Junta de Andalucía, authorized the sampling of invertebrates in wetlands and the collection of deserted eggs. We thank Manolo Vázquez and Inma Cintado for their help in preparing egg and prey samples, respectively.

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