

# Nine

## Seasonal carry-over effects: feather isotope signatures of diet during spring stopover correlate with female arrival at breeding site, body mass and egg volume in black-tailed godwits

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### Abstract

Over the last five decades black-tailed godwits (*Limosa limosa*) that breed in temperate grasslands have experienced great changes in habitat during their spring stopover en route from West-Africa to The Netherlands. They spend winter and spring staging mostly on rice fields where they only eat rice grains. Few birds also forage on natural fresh-water wetlands of marine mudflats. Different diets may lead to differential resource allocation during prealternate molt and migration, which in turn may affect reproduction. We used stable isotopes in feathers of black-tailed godwits from a breeding population in The Netherlands to detect a possible influence of diet on timing of arrival on the breeding grounds, on body mass during late incubation, and on correlates of reproductive success (egg volume and timing of breeding). Female godwits had lower  $\delta\text{C}$ , but not  $\delta\text{N}$ , signatures than males. Female arrival time, body mass but not size, and egg volume were positively correlated with  $\delta\text{N}$ , but not with  $\delta\text{C}$ . Although we cannot conclude the prey type, the quality of prey items on the spring staging areas affects reproductive success, both directly and indirectly.

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## Introduction

MANY MIGRATORY BIRDS spend most of their lifetime in places thousands of kilometers away from their natal and breeding sites, and the conditions on the staging sites may affect the fitness of individual birds (Cristol, 1995; Kokko, 1999; Bêty *et al.*, 2003; Bêty, Giroux & Gauthier, 2004). In detail, such carry-over effects are mediated through food abundance on staging areas (Ebbinge & Spaans, 1995; Marra *et al.*, 1998; Norris *et al.*, 2003). A first step towards understanding carry-over effects (Greenberg & Marra, 2004) incorporates analyses of food abundance and/or quality on the staging grounds and how these correlate with reproductive indices on the breeding grounds (Ebbinge & Spaans, 1995; Marra *et al.*, 1998; Norris *et al.*, 2003).

Black-tailed godwits (*Limosa limosa*), a migratory shorebird, provide an interesting case as over the last half-century many natural wetlands on the Iberian Peninsula used as spring staging areas were replaced by rice fields during the last century (Lains & Sousa, 1998; Kuijper *et al.*, 2006). Currently the main food during spring staging at the Iberian Peninsula consists of spilled rice (Sánchez-Guzmán *et al.*, 2007; Lourenço & Piersma, 2008a), while a smaller part of the populations utilizes natural fresh-water wetlands and mudflats (Kuijper *et al.*, 2006). Godwits of the nominate race (*L. l. limosa*) are currently suffering from a strong population decline at a rate approaching 30% over the last 15 years (Thorup, 2006; Gill *et al.*, 2007). A reduction in adult survival does not seem to be involved and the most common explanation for the decrease is low breeding success (Beintema *et al.*, 1995; Gill *et al.*, 2007; Roodbergen *et al.*, 2008; Hötker, Jeromin & Melter, 2007; Schekkerman *et al.*, 2008). Conditions on the spring staging grounds may contribute to these declines if different diets affect the timing of northward migration and breeding as well as body condition with cascading effects on reproductive success.

In the very closely related subspecies (Höglund *et al.*, 2009), the Icelandic black-tailed godwit (*L. l. islandica*), evidence for carry-over effects have actually been found. Individuals wintering in high quality saline areas arrive on the Icelandic high quality breeding grounds earlier than birds wintering in low quality freshwater areas (Gunnarsson *et al.*, 2006b). Further, birds that foraged during winter on more marine food were breeding in high quality areas and had a higher reproductive success than birds foraging on freshwater prey (Gunnarsson *et al.*, 2005b). Here we aim to explore the links between isotopic indices of spring staging diets and measures of breeding performance in individual black-tailed godwits breeding in the northern Netherlands. To do so, we study the relationships between stable carbon and nitrogen isotopic signatures of breeding feathers produced during spring staging in Iberia and the timing of arrival on the breeding grounds, body mass and size during late incubation as well as laying date and egg volume.

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## Methods

### Field work

Data were collected from 2005–2007 in the Workumerwaard, Friesland, The Netherlands. For a detailed description of the study area see Schroeder *et al.* (2008a) and van den Brink *et al.* (2008). We visited godwit nests in the beginning of the incubation period and measured the length and width of eggs. A few days before the expected hatching date, we caught birds with walk-in traps on the nests. We weighed the birds to the nearest g and measured wing length (flattened and straightened,  $\pm 1$  mm), bill length (exposed culmen,  $\pm 0.1$  mm), total head length ( $\pm 1$  mm), tarsus length ( $\pm 0.1$  mm), and tarsus+toe length (tarsus plus mid-toe length without nail  $\pm 1$  mm). With scissors, we cut one breeding feather from the back of each adult bird. On the following days, nests were checked daily for hatching success.

A blood sample of 20  $\mu$ l was taken from the brachial wing vein and stored in 96% ethanol. DNA was extracted in the laboratory by the chelex extraction method (Walsh *et al.*, 1991). PCR products were acquired using the method of Griffiths *et al.*, (1998). Instead of an agarose gel, we separated the PCR products on an ABI 377 automatic sequencer and their length was determined with Genescan 3.1 software. Bird sexes were scored following Schroeder *et al.* (2008a).

We used average egg volume per nest as a component of reproductive success. Black-tailed godwits, like most waders, have an invariant clutch size and the correlation between egg volume and chick mass at hatching is very strong (own data). Egg volume is linked to hatchling mass, which in turn is linked to fledging success and thus egg volume can be used as a proxy for reproductive success (own data). Laying date and hatching date were calculated using the angle in which an egg flows in water (van Paassen *et al.*, 1984; Liebezeit *et al.*, 2007). Whether or not a nest successfully hatched was used as hatching success (binary variable).

Resightings of individually color-ringed birds have been made in January and February of 2005 and 2006 in Portugal and Spain on the main staging sites. The sites in Portugal, located alongside the Sado (38°24'N, 8°38'W) and Tejo (38°57'N, 8°54'W) estuaries, are rice fields. Godwits normally foraged on the rice fields and rested on the mudflats (a description of the areas can be found in Lourenço & Piersma, 2008a). From Portugal, it is known that godwits forage up to 94% on rice, and estimated 6% on animal prey items (Lourenço & Piersma, 2008a). The staging sites in inland Spain are approximately 300 kilometers west of the Portuguese sites, rice fields close to the village Hernán Cortéz in Extremadura, Spain (30°01'N, 5°55'W). There, rice fields represent the only suitable habitat for godwits and they are only seen there (for a description of the area we refer to Sánchez-Guzmán *et al.*, 2007). Here, godwits forage nearly exclusively on rice (J.A.M. & F.S.Q., unpublished data). We therefore expect the isotopic values to differ between the two sites with respect to trophic level and the closer proximity of estuarine prey in Portugal.

Arrival time on the breeding grounds was assessed by searching the breeding area for color-marked individuals from 1 March to 1 May 2005, 2006 and 2007, at least

three times per week. The daily resighting probability of individuals from the date of the first observation until the start of breeding was  $0.37 \pm 0.016$  SE (N = 2235, based on 134 color-ringed individuals). The date a bird was first observed in a given year was used to indicate arrival time.

### Stable isotopes

Stable isotopes are naturally occurring stable forms of elements, which differ in their nuclear masses. Stable isotopes are assimilated directly with the food and become fixed in the tissue during growth, for instance in a feather. Once made, feathers are metabolically inert. The stable isotope ratio remains; reflecting the composition of food at the time and place during which they were grown (Mizutani *et al.*, 1990; Hobson, 1999; Hobson & Clark, 1992; Rubenstein & Hobson, 2004). Since many migratory birds molt during spring migration, this offers a relatively low-effort method to get more information on the geographic origin of the birds (Hobson, 1999; Pain *et al.*, 2004; Rubenstein & Hobson, 2004; Yohannes *et al.*, 2006) and the possibility to link food quality on spring staging grounds to individual breeding success (Rubenstein *et al.*, 2002; Webster *et al.*, 2002; Norris *et al.*, 2003). We used two different isotopes: nitrogen and carbon.

We used breeding feathers from the back of Black-tailed godwits as sample material. These feathers are grown on the staging sites in January and February, when they molt into their breeding plumage (Cramp & Simmons, 1983; J.S. & P.M.L. pers. obs.). We additionally measured isotope ratios in prey items. Rice and invertebrate prey items have been collected in the study area (rice fields) in Portugal in 2006 and in the study area in Spain in 2007. Animal prey items were not sampled in the Spanish rice fields because densities were low.

Before further processing, the samples have been cleaned from surface contaminations using 96% ethanol and following this 99% hexane. Then, 200 $\mu$ g of the feathers were analyzed for carbon stable isotope ratio (parts per thousand, ‰, difference from the  $^{13}\text{C}/^{12}\text{C}$  ratio in Vienna PeeDee limestone; further referred to as  $\delta^{13}\text{C}$ ) and nitrogen stable isotope ratio (‰ difference from the  $^{15}\text{N}/^{14}\text{N}$  ratio in atmospheric  $\text{N}_2$ ; further referred to as  $\delta^{15}\text{N}$ ) in a HEKAtech EuroEA elemental analyzer coupled on-line through a Finnigan con-flo interface to a Finnigan Delta S isotope ratio mass spectrometer. Reproducibility based on replicate measurements on a casein standard (N = 145) during the period of measurements was 0.12‰ (=SD) for both elements.

### Statistical analyses

All body size measurements of black-tailed godwits (not including body mass) were collapsed in a principle component analysis. The first principal component (PC1) explained 77.72% of variation and is used in the following as variable “size”. Birds with a higher value of PC1 are larger than birds with a lower value. We used residuals of a linear regression of body mass on size as size-corrected body mass of the birds ( $R^2 = 0.53$ ;  $F_{1,78} = 86.99$ ;  $P < 0.001$ ), further referred to as condition. We did not have size measurements of three birds and those were excluded from the analysis of size and condition.

Data on  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  were not normally distributed; with a log transformation of the absolute values we achieved normality. Body mass was standardized before statistical analysis. Average egg volume and laying dates were standardized per year to account for between-year variation. Repeatabilities were calculated following Lessells & Boag (1987).

We performed general linear models (GLMs) and included a factor for sex (females code as 0, males as 1) in all models. The godwits received individual color-rings on capture. Since we do not have sufficient data on first observation dates for the same season where we have data on stable isotopes we use the first observation dates in the subsequent year after catching for analysis, assuming that birds are repeatable in their diet on the staging grounds. This is a valid assumption since repeatabilities of isotopic signatures were quite high (see below). Because the carry-over effect may differ between the sexes, we tested for the interaction between sex and isotope signature. To account for between-year variation, we added a fixed factor coding for year to the most parsimonious models. Body mass, condition and egg volume might vary over the course of the season. To account for this we added laying date as a covariate to the most parsimonious models explaining these variables. Heavier females are known to lay larger eggs (own data). Therefore we added body mass as covariate to the most parsimonious models explaining variance in average egg volume. Since birds with a higher  $\delta^{15}\text{N}$  have a higher body mass (this study), which in turn is expected to influence egg volume, we included the interaction between body mass and  $\delta^{15}\text{N}$  to the model.

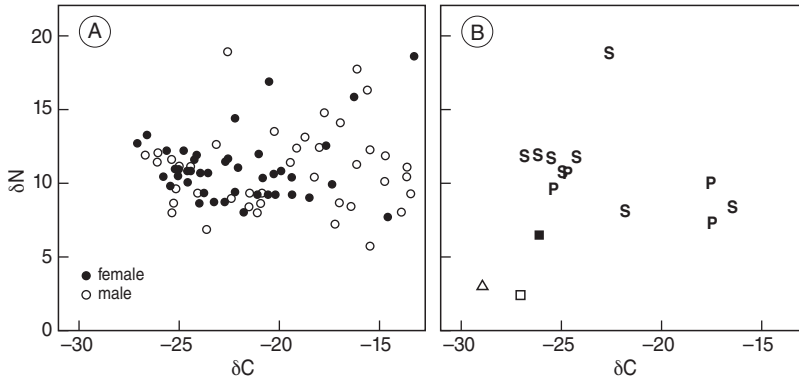
We used R.2.7.1 statistical software (R Development Core Team, 2008) and the `lm()` function of the base package for fitting linear models (Crawley, 2007). For fitting the logistic model we used the `glm()` function from the base package with the binomial link function. We used Akaike's information criterion to select the most parsimonious model employing the `stepAIC()` function from the base package (Akaike, 1973; Burnham & Anderson, 2002). We report ANOVA result tables for the models we tested, and parameter estimates with standard errors where all variables from the most parsimonious model and the correction factors year and laying date were in the model.

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## Results

WE COLLECTED DATA on stable isotope signatures of 40 male and 43 female black-tailed godwits breeding in The Netherlands in the years 2005-2007. Four female godwits were sampled in two years and between-year repeatability of the isotope values was high in  $\delta^{15}\text{N}$  ( $0.56 \pm 0.37$  SE; ANOVA:  $F_{3,7} = 4.73$ ;  $P = 0.08$ ) and very high in  $\delta^{13}\text{C}$  ( $0.86 \pm 0.14$  SE; ANOVA:  $F_{3,7} = 13.08$ ;  $P = 0.02$ ).

Male godwits breeding in The Netherlands had on average higher  $\delta^{13}\text{C}$  values than females (Fig. 9.1A, males:  $-20.02 \pm 0.68$  SE; females:  $-22.55 \pm 0.44$  SE;  $t = -3.12$ ,  $P = 0.003$ ,  $N = 83$ ). There was no difference in  $\delta^{15}\text{N}$  values between the sexes (Fig. 9.1A, males:  $10.59 \pm 0.31$  SE; females:  $10.98 \pm 0.33$  SE;  $t = 0.73$ ,  $P = 0.47$ ,  $N = 83$ ).

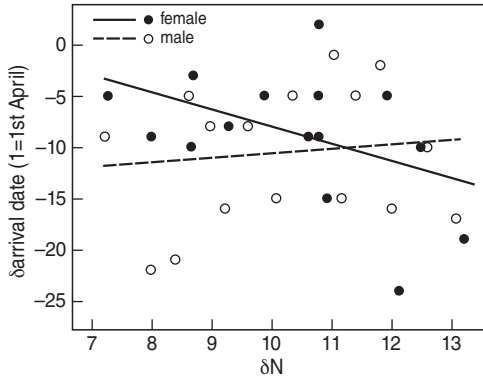


**Figure 9.1:**  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  measured in (A) breeding feathers of black-tailed godwits breeding in The Netherlands. Solid circles are female, open circle male godwits. (B)  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  measured in potential prey items in Iberia. The triangle depicts isotopic signature of animal prey items from Portugal, open squares depicts rice grains from Portugal, the filled square rice grains from Extremadura, Spain. Isotope measurements of breeding feathers of godwits resighted in the region are printed, S=resighted in Spain, P=resighted in Portugal.

Rice sampled in Spain ( $N = 2$ ) had higher  $\delta^{15}\text{N}$  and a comparable  $\delta^{13}\text{C}$  value than rice from Portugal ( $N = 4$ ) (Fig. 9.1B, rice  $\delta^{15}\text{N}$ : Spain =  $6.4 \pm 0.3$  SD; Portugal =  $2.38 \pm 0.70$ SD;  $\delta^{13}\text{C}$ : Spain =  $-26.13 \pm 0.19$  SD; Portugal =  $-27.03 \pm 0.64$  SD). Portuguese animal prey items ( $N = 2$ ) had isotope signatures similar to those of Portuguese rice (Fig. 9.1B;  $\delta^{15}\text{N} = 3.07 \pm 1.16$ ;  $\delta^{13}\text{C} = -28.94 \pm 0.24$  SD). We observed 12 individuals in Iberia during spring migration for which we had collected feather samples: eight in Spain and four in Portugal. Their stable isotope values did not differ between staging areas or sex (Fig. 9.1B;  $\delta^{15}\text{N}$  mean Portugal = 9.37, mean Spain = 11.66;  $\delta^{13}\text{C}$  mean Portugal =  $-23.55$ , mean Spain =  $-21.12$ ; Anova:  $\delta^{13}\text{C}$ :  $F_{\text{sex}} = 0.01$ ,  $P = 0.92$ ,  $F_{\text{location}} = 1.04$ ,  $P = 0.33$ ,  $df = 2,9$ ;  $\delta^{15}\text{N}$ :  $F_{\text{sex}} = 0.09$ ,  $P = 0.77$ ,  $F_{\text{location}} = 1.67$ ,  $P = 0.23$ ,  $df = 2,9$ ).

On the basis of first observation dates of 17 male and 16 female godwits for which data on stable isotopes were known, average first observation date of these birds occurred on 21 March  $\pm 1.11$  day SE, and it did not differ between the sexes or years (Factorial ANOVA:  $F_{\text{sex}} = 1.00$ ,  $P = 0.32$ ,  $F_{\text{year}} = 2.23$ ,  $P = 0.14$ ,  $df = 2,30$ ). Females with the highest  $\delta^{15}\text{N}$  arrived on average 20 days earlier on the breeding grounds than females with the lowest  $\delta^{15}\text{N}$  (Fig. 9.2), while there was no correlation in males (Table 9.1). We found no statistical effect of  $\delta^{13}\text{C}$ ; the interaction between  $\log -\delta^{13}\text{C}$  and sex and  $\log -\delta^{13}\text{C}$  did not remain in the final model (Table 9.1).

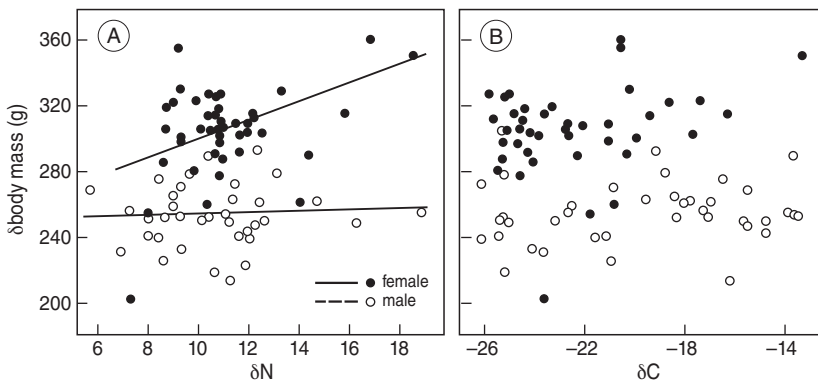
Birds with a higher  $\delta^{15}\text{N}$  were heavier. This effect was stronger in females than in males, and the interaction of  $\log -\delta^{15}\text{N}$  with sex remained in the most parsimonious model (Fig. 9.3A, Table 9.2).  $\log -\delta^{13}\text{C}$  and the interaction of  $\log -\delta^{13}\text{C}$  and sex were removed from the final model (Table 9.2). Size was not explained by any of the isotopic signatures. All effects were removed from the final model but sex ( $F_{\text{sex}} = 184.34$ ,  $P < 0.001$ ,  $df = 1,78$ ; all other effects  $P > 0.32$  before removal). In the most parsi-



**Figure 9.2:** First observation date dates of female and male black-tailed godwits on their breeding grounds in The Netherlands in relation to the  $\delta^{15}\text{N}$  signature of their back breeding feathers. Straight line is a regression line for females, dotted line for males. See Table 8.1 for statistics.

**Table 9.1:** Results of a GLM with first observation date (arrival) of Black-tailed godwits breeding in The Netherlands as dependent variable. AIC was used for the decision to reject effects.

Variable	F <sub>4,28</sub>	P	Estimate $\pm$ SE
Sex	1.57	0.33	-63.43 $\pm$ 29.25
Year	2.57	0.12	-6.28 $\pm$ 2.54
Log(dN)	1.87	0.18	-25.14 $\pm$ 9.84
Log(dN) x sex	4.35	0.05	26.24 $\pm$ 12.58
Rejected effects			
Log(-dC)	0.53	0.47	-4.41 $\pm$ 5.76
Log(-dC) x sex	1.44	0.24	17.79 $\pm$ 14.82



**Figure 9.3:** Body mass of female (bullets) and male (open circles) black-tailed godwits breeding in The Netherlands and in relation to (A)  $\delta^{15}\text{N}$  and (B)  $\delta^{13}\text{C}$  signature of their back breeding feathers. Straight line is for females, dotted line for males. For statistics see text and Table 8.2.

**Table 9.2:** Results of a GLM with body mass of Dutch Black-tailed godwits as dependent variable.

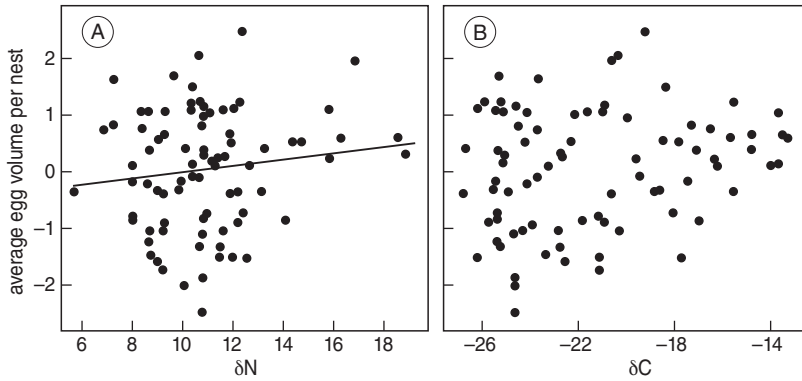
Variable	F <sub>5,77</sub>	P	Estimate ±SE
Sex	44.11	<0.001	2.79 ±1.69
Year	2.47	0.11	-0.16 ±0.16
Laying date	0.01	0.92	0.002 ±0.009
Log(dN)	14.48	<0.001	1.93 ±0.57
Log(dN) × sex	6.31	0.01	-1.80 ±0.71
Rejected effects			
Log(-dC)	1.80	0.18	-0.40 ±0.40
Log(-dC) × sex	0.10	0.75	0.28 ±0.91

**Table 9.3:** Results of a GLM with size-corrected body mass (condition) of Dutch Black-tailed godwits as dependent variable.

Variable	F <sub>5,74</sub>	P	Estimate ±SE
SSex	2.45	0.12	3.93 ±1.12
Year	1.52	0.22	-0.13 ±0.24
Laying date	0.52	0.47	0.01 ±0.01
Log(dN)	2.22	0.14	1.97 ±0.92
Log(dN) × sex	2.62	0.11	-1.81 ±1.12
Rejected effects			
Log(-dC)	0.96	0.33	-0.47 ± 0.61
Log(-dC) × sex	0.001	0.97	0.04 ± 1.37

monious model explaining variance in condition, again the interaction between log  $\delta^{15}\text{N}$  and sex remained in the model, but this effect was not statistically significant (Table 9.3). Log  $-\delta^{13}\text{CC}$  and the interaction of log  $-\delta^{13}\text{C}$  and sex were removed from the final model (Table 9.3).

Egg volume was higher in nests that were incubated by birds with a higher  $\delta^{15}\text{N}$ . This effect was on mainly due to a higher body mass in birds with a higher  $\delta^{15}\text{N}$ : the interaction of log  $-\delta^{15}\text{N}$  and body mass remained (Table 9.4). There was a non-significant trend for nests that were incubated by birds with a higher  $\delta^{13}\text{C}$  to contain larger eggs, independent of body mass (Table 9.4, Figure 9.4). We found no relationship between any of the isotopes and laying date, and all effects were removed from the most parsimonious model (all effects  $P > 0.30$  before removal). Hatching success was not related to isotope value, and no variable remained in the most parsimonious model (binomial logistic model:  $N = 89$ , log  $-\delta^{15}\text{N}$ : Wald  $Z = -0.48$ ,  $P = 0.63$ ; log  $-\delta^{13}\text{C}$ : Wald  $Z = -0.13$ ,  $P = 0.89$ ).



**Figure 9.4:** Average egg volume per nest standardized per year of black-tailed godwits breeding in The Netherlands in relation to (A)  $\delta^{15}\text{N}$  and (B)  $\delta^{13}\text{C}$  signature of their back breeding feathers. For statistics see text and Table 8.4

**Table 9.4:** Results of a GLM with standardized egg volume of Dutch Black-tailed godwits as dependent variable.

Variable	$F_{5.77}$	P	Estimate $\pm$ SE
Year	1.23	0.27	$-0.27 \pm 0.24$
Laying date	0.18	0.67	$-0.01 \pm 0.01$
Body mass	3.20	0.07	$-2.73 \pm 1.08$
$\text{Log}(\delta^{15}\text{N})$	0.52	0.47	$0.73 \pm 0.57$
$\text{Log}(\delta^{15}\text{N}) \times \text{body mass}$	5.55	0.02	$1.06 \pm 0.45$
Rejected effects			
$\text{Log}(-\delta^{13}\text{C})$	2.29	0.13	$-0.75 \pm 0.60$
sex	0.19	0.66	$0.27 \pm 0.36$
$\text{Log}(-\delta^{13}\text{C}) \times \text{sex}$	1.20	0.28	$-1.10 \pm 0.28$
$\text{Log}(\delta^{15}\text{N}) \times \text{sex}$	1.08	0.30	$1.67 \pm 1.68$
$\text{Log}(\delta^{15}\text{N}) \times \text{sex} \times \text{body mass}$	0.01	0.92	$-0.19 \pm 1.90$

## Discussion

WHATEVER DIET COMPONENT yielded high  $\delta^{15}\text{N}$  feathers correlated with female arrival, body mass during incubation and egg volume. What could these high  $\delta^{15}\text{N}$  values represent? Nitrogen isotopes undergo trophic level discrimination and can therefore be used as dietary markers indicating the relative trophic level of the food the animal ingested (Thompson, Furness & Lewis, 1995; Kelly, 2000). However, the difference in nitrogen isotopic signatures between rice and animal prey items collected in Portugal was considerably smaller than the difference between rice collected in

Extremadura and in Portugal, preventing us from making strong statements about the trophic level of the food ingested (Fig 9.1B). That rice from Spanish staging areas had a higher  $\delta^{15}\text{N}$  value than rice from Portuguese staging grounds might be due to a differential use of fertilizers: plants growing on soil fertilized with organic manure have a considerably higher  $\delta^{15}\text{N}$  signature than plants growing on soil fertilized with artificial fertilizers (Denton *et al.*, 2001). Visual inspection of the isotope ratios of individuals resighted only in Spain indeed clustered towards higher  $\delta^{15}\text{N}$  compared with birds only resighted in Portugal (Figure 9.1B). However, given the dual effects of degree of animal (estuarine) matter and nitrogen fertilizer on  $\delta^{15}\text{N}$  signatures, we cannot distinguish godwits foraging on rice grains and animal prey items.

Isotopic signatures of  $\delta^{13}\text{C}$  can indicate the degree of freshwater and marine habitat use with more negative values indicating a higher ratio of freshwater food and higher values suggest higher proportions of estuarine food items (Klaassen *et al.*, 2001; Gunnarsson *et al.*, 2005b). Surprisingly, however, the Portuguese food samples from estuarine areas had somewhat lower  $\delta^{13}\text{C}$  values than the Spanish ones (Fig. 9.1B) although the differences were not statistically significant. As it is likely that Portuguese rice fields were irrigated with upstream river water, we suggest that the high  $\delta^{13}\text{C}$  of the Portuguese birds might stem from marine prey items they may have ingested on nearby estuarine mudflats. The highest  $\delta^{13}\text{C}$  signature comes from a Spanish bird, which suggests that this bird might have foraged on marine prey somewhere at the coast (e.g. in Portugal) before it was resighted in inland Extremadura. Our resighting data and carbon stable isotopes suggests that at least some godwits do forage on a variety of staging sites and those are most likely on a mixed diet that also includes marine prey. It is not clear yet where they ingest this marine prey, and next to the Portuguese coast it is likely that birds make use of other sites, like the Doñana Wetlands. This is an important staging site, but we did not have resightings nor prey samples from there (Rendon *et al.*, 2008). Overall, we do not find evidence for spatial segregation of staging godwits.

We showed that signatures for  $\delta^{13}\text{C}$  differ between sexes. Females forage on average on food items with lower  $\delta^{13}\text{C}$  values than males (Fig. 9.1A). This could indicate that females forage mainly on rice fields, while part of the males forage supplementary on prey with higher carbon isotope signatures, most likely marine prey (Fig 9.1A). Black-tailed godwits caught on Spanish rice fields did also show a similar difference between sexes in  $\delta^{13}\text{C}$  measured of toenails and blood (JAM & FSQ, unpublished data). Because in that case, both sexes were caught on the same place, a spatial separation of genders is less likely. It may be that the timing of molt differs between the sexes, which could lead to such a divergence, but we have no evidence to prove this. This issue is clearly not solved yet and to do so we need more information on gender based food intake and migration schedules.

Although we cannot disentangle habitat and food sources, we do find evidence for a seasonal carry-over effect: Female birds that consume prey items with a higher  $\delta^{15}\text{N}$  during wintering and spring staging arrived earlier on the breeding grounds and were heavier, but not larger and laid larger eggs than females foraging on sites and prey items resulting in a lower  $\delta^{15}\text{N}$  (Figure 9.2A). These effects may be linked with each other: if

a female manages to be early on the breeding ground she has more time to gather nutrients needed to produce eggs, and can be fatter during incubation. Females heavier for their size were found to lay larger eggs, which could explain the correlation between the nitrogen isotope signatures and egg volume (Schroeder *et al.*, 2009). We did not find an effect of isotope signature on timing of arrival in males. This is surprising because theory suggests that early arriving males have an advantage in securing high quality territories, which in turn is expected to positively influence female mate choice and ultimately fitness (Kokko, 1999, Gunnarsson *et al.*, 2006b). One possible explanation is that as the differentiation in carbon signatures suggests, males and females may molt at different times, resulting in isotopic signatures that reflect the nutritional situation of different times for each sex. If this would be true, it would also suggest that the nitrogen isotopic value of males says a different thing than that of females.

We did not find significant effects of the carbon isotope signatures on individual traits on the breeding grounds, which is surprising since Gunnarsson *et al.* (2005b) found that birds that wintered in more saline habitats that resulted in higher carbon isotopic values breed in better quality areas and have higher reproductive output than birds wintering in inland areas that result in lower carbon isotope values. This study was based on averages of different godwit breeding areas, while we study individual traits (Gunnarsson *et al.*, 2005b). We did find a trend for birds with higher carbon signatures to incubate larger eggs, and it may be that this pattern would become more pronounced if we were to study it on the level of breeding areas differing in quality.

In conclusion, although we do not find evidence for spatial segregation, we do find support for the idea that female and male godwits differ in their prey or location choice on the spring staging grounds. Further, the choice of location and prey affects reproductive correlates on the breeding grounds, mostly in females. Since our results are mere correlations we cannot infer any causal relations and direction, - it may be that females of higher quality forage on higher quality prey items because they are of higher quality or that foraging on high quality prey makes a female better.

We suggest that data on resightings of color ringed individuals on other possible staging areas (Cota Doñana, Carmargue, Cádiz Salinas), together with an analysis of food items of these sites, could give a better insight to how different habitats and diets during spring staging affect reproductive output in the nominate race of the black-tailed godwit. Since these carry-over effects affect population dynamics it is important to take them into account when thinking about how to stop the ongoing population decline of the Black-tailed godwit in The Netherlands.

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