Tracking variations in wetland use by breeding flamingos using stable isotope signatures of feather and blood

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ABSTRACT

This study tracks temporal variations in the habitat use of breeding adult greater flamingos (*Phoenicopterus roseus*) in the Camargue (southern France) using simultaneous sampling of $\delta^{15}\text{N}$, $\delta^{13}\text{C}$ and $\delta^{34}\text{S}$ from feather and blood of chicks at fledging. Camargue flamingos forage in a heterogeneous complex of saltpans, permanent and temporary brackish lagoons, freshwater and euryhaline marshes to provision their chicks over a two month period. Using Bayesian mixing models with diet and tissue specific discrimination factors and invertebrates collected from 23 locations, we investigated whether blood and feather isotopes indicated temporal variations in habitat use relative to salinity and hydroperiod. We also tested whether fledgling body condition could be explained by the isotopic signatures of their tissues. While $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values did not differ significantly between blood and feather, marked differences were apparent in the $\delta^{34}\text{S}$ values obtained from these tissues. Saltpans (38%) and freshwater marshes (33%) appeared to be the main habitats visited by adult birds in the early phase of parental care with use of saline wetlands increasing later in the season (54%). This habitat shift may be related to the peak of resources in saltpans and the drying up of freshwater and brackish marshes by mid summer. Habitat shift (as expressed in individual shifts in isotope values between feather and blood) was not correlated with offspring age, and differed between individuals. A negative relationship was observed between offspring body condition and feather $\delta^{15}\text{N}$, indicating that chicks fed from temporary flooded marshes fare better than those provisioned from permanent marshes. Foraging strategies of greater flamingos during parental care were heterogeneous, possibly tracking changes in resource availability as the season progressed and reflecting differences in the competitive ability of parent birds. Given that the Camargue saltpans face closure and the region's temporary wetlands remain threatened, our results emphasize the critical importance of conserving these two key habitats for greater flamingos. Dual tissue, triple stable isotope analysis provides a useful and sensitive means of tracking localized environmental change in this threatened system.

Keywords:
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index of isotopic shift
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1. Introduction

Habitat shifts during different life history stages are known to be commonplace among fish (Muñoz and Ojeda, 1998), including freshwater (e.g. Schleuter and Eckmann, 2008), estuarine (e.g. Jackson and Rundle, 2008) and reef (e.g. Wells et al., 2008) species. Such shifts are generally thought to be adaptive strategies to minimize intraspecific competition (Werner and Gilliam, 1984) or to support ontogenetic changes such as growth (Ward Campbell and Beamish, 2005). Similar shifts in resource use are also observed in other species where offspring are supported by

parental investment, including birds. After chicks have hatched, their increasing energy demands lead to greater food (nutritional) requirements. Parents that feed their offspring must respond to these changes either by increasing the quantity of food supplied (Winkler, 1987; Emms and Verbeek, 1991) or by shifting to more profitable foods (e.g. Cairns, 1987).

Identifying shifts in foraging behaviour during different stages in the reproductive process may have important consequences for delineating habitat use and prioritizing conservation areas (Ramirez et al., 2011; Brittain et al., 2012). However, direct estimation of habitat use by breeding birds is difficult. Tracking adult birds on their foraging trips usually requires either telemetric methods (Amat et al., 2005) or traditional and time consuming approaches such as marking and resighting (e.g. Béchet et al., 2009).

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An alternative approach to tracking habitat use by breeding birds is to monitor stable isotope ratios of carbon ($\delta^{13}\text{C}$), nitrogen ($\delta^{15}\text{N}$) and sulphur ($\delta^{34}\text{S}$) in multiple tissues obtained from dependent chicks (Quillfeldt et al., 2008). Carbon isotope ratios ($\delta^{13}\text{C}$) reflect the source of primary productivity and can be used as indicators of an animal's habitat utilization as well as distinguishing the nutritional composition of diet (e.g. Rubenstein and Hobson, 2004). Enrichment of $\delta^{15}\text{N}$ with increasing trophic levels provides a means of estimating an organism's relative trophic position (Peterson and Fry, 1987). Sulphur isotope ratios ($\delta^{34}\text{S}$) provide information on dietary protein source and geographical origin (Richards et al., 2003) and have been used to identify patterns relating to the salinity of feeding locations (e.g. Fry and Chumchal, 2011). As dietary nutrients are incorporated at different times depending on growth, metabolism and protein turnover of the tissue considered, different tissues from the same individual may provide information on dietary source at different phases of life history. In the case of birds, whole blood (hereafter blood) is an active tissue and its isotopic signature may represent diet integrated over weeks to about a month (Bauchinger and McWilliams, 2009), while feather is inert keratin tissue that reflects dietary sources used during feather formation. Analysis of simultaneously acquired samples can therefore reveal both spatial and temporal variations in diet.

This study aimed to investigate temporal variation in habitat use by breeding greater flamingos (*Phoenicopterus roseus*), a flagship species for the conservation of Mediterranean wetlands (Johnson and Cézilly, 2007; Béchet et al., 2012). During incubation and early chick rearing, breeding adults forage in heterogeneous and complex habitat patches with varying salinity gradients and hydroperiods (Béchet et al., 2009). Chicks are fed by parents for an extended period, being unable to feed by themselves for up to 75 days after hatching, as their bill (which is used as a filter apparatus for feeding) is not sufficiently developed (Jenkin, 1957). At this stage, parents do not regurgitate food for their young, but feed them with secretions from glands located in the digestive tract (Lang, 1963). Young birds fly and become independent 2–3 months (75–90 days) after hatching. Summer drying of marshes during the rearing season may enforce a change in habitat use to effectively exploit available resources. Furthermore, qualitative and quantitative changes in the dietary needs of developing chicks may trigger temporal variation in adult habitat use. Sex specific habitat preferences in adults could also lead to temporal variation in chick dietary provenance, because as chicks get older, male parents typically increase the time spent feeding their offspring, while females do not (Cézilly et al., 1994).

The current study sought to test a hypothesis based on the above observations, that greater flamingos raising offspring exhibit habitat shift. Stable isotope signatures were obtained from samples of two tissue types with varying isotopic turnover rate, namely blood and feather, taken from chicks shortly before fledging. It was expected that feather and blood isotope signatures would allow for the comparison of early (~first month) versus late (~second month) habitat use by parent birds. However, differences in isotopic composition between diet and consumer tissues, also known as discrimination factors (or fractionation factors), are known to restrict direct interpretation of tissue stable isotopes. Usually, discrimination factors are tissue and diet specific (Caut et al., 2009), so applying them independent of tissue or diet isotopic values can bias the interpretation of the results and lead to erroneous conclusions. For this reason, diet and tissue specific discrimination factors were applied to the analyses, as described in Caut et al. (2009), allowing habitat use of breeding greater flamingos to be tracked using simultaneous sampling of feather and blood $\delta^{15}\text{N}$, $\delta^{13}\text{C}$ and $\delta^{34}\text{S}$.

The following predictions were made:

- 1 if parental habitat use tracks changes in resource availability such that offspring are provisioned from seasonally variable dietary sources, there should be a detectable shift (both in magnitude and direction) in most of the individuals examined, i.e. habitat shifts are adaptive responses to changes in resource availability;
- 2 if foraging habitat shifts are related to morphological changes in chicks (e.g. growth and physiological maturation), then a strong correlation between chicks age and tissue stable isotope signatures is to be expected, i.e. habitat shifts are adaptive responses to ontogenetic changes in offspring, such as growth.

This study used triple isotopic models with 5 endpoints of salinity gradient and 3 hydroperiod to investigate whether variations in blood and feather stable isotopes indicate population level variations in habitat use with varying salinity and hydroperiod.

2. Materials and methods

2.1. Study species and chick sampling

Greater flamingos have bred intermittently in the brackish lagoons of the Camargue for centuries (Johnson and Cézilly, 2007). Since 1974, successful breeding has taken place on a man made island on the Fangassier lagoon (43°25'N, 4°37'E), part of the 11,000 ha commercial salt pans of Salin de Giraud (Fig. 1). A single egg is usually laid in April and the laying period is spread over 30–74 days. Both parents incubate and feed the chick and incubation lasts 29 days. When chicks are about three weeks old, they form a crèche (Tourenq et al., 1995) where they remain until fledging, 75–90 days after hatching.

In 2010, 45 flamingo chicks were caught just prior to fledging on day 79 (4th of August) after first egg hatching. The following year, a further 12 chicks were captured on day 66 (27th of July 2011). Each bird was ringed with a band bearing a unique alphanumeric code. Tarsus length was measured and body weight determined using a 0–5000 g Pesola spring balance. From each chick, a few body feathers were collected and stored dry until required for stable isotope analyses. Blood (300–500 μl) was taken from 22 of the 45 birds captured in 2010 and 8 of the 12 birds taken in 2011. Samples were drawn from a vein in the leg soon after capture and kept frozen (-80°C) until required for laboratory analysis.

2.2. Invertebrate sampling

In July 2011, samples of greater flamingo prey were collected from 23 adult flamingo foraging locations (Fig. 1; Béchet et al., 2009). In order to minimize bias that could be generated if a mixture of invertebrate prey species were to be sampled at each site, this study focused on one taxon, namely oligochaetes. Oligochaetes are abundant across the region's wetlands and their isotopic signature is believed to be broadly representative of the habitats they are collected from. Their signatures should therefore allow tracking of parental habitat use during feather and blood cell synthesis in flamingo chicks.

2.3. Salinity and hydroperiod

Salinity and hydroperiod are key factors shaping the invertebrate communities of the Camargue wetlands (Waterkeyn et al., 2008). The salinity (as the practical salinity scale) of each sampled wetland was measured *in situ* in July 2011 by electric conductivity, and varied from 3.6 in freshwater marshes to 114 in

Fig. 1. Greater flamingo breeding colony (white star) and sampling sites (temporary (○), semi-temporary (◐) and permanent marshes (●) along the salinity gradient, classes 1 to 5; see text for details) at their main foraging areas within the Camargue wetlands complex.

concentrated salt pans. We defined 5 salinity classes based on changes of macrophyte communities, an important criterion used to describe wetland types for habitat conservation:

- Class 1, salinity 0–5: freshwater species (e.g. *Myriophyllum spicatum*),
- Class 2, salinity 5–10: halotolerant species (e.g. *Zannichellia pedunculata*),
- Class 3, salinity 10–30: halophile species of brackish wetlands (e.g. *Chara canescens*),
- Class 4, salinity 30–40: halophile species of salty wetlands (e.g. *Ruppia* sp., *Zostera noltii*) and
- Class 5, salinity 40–120: salt pans with little or no macrophyte vegetation.

Based on the duration of flooding and irrigation drainage in puts, wetlands were further assigned to one of three hydroperiod groups: temporary, semi temporary (with potential for increases flood duration due to water input from irrigation drainage) and permanently flooded marshes.

2.4. Laboratory analysis

We analyzed stable $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ and $\delta^{34}\text{S}$ in blood, feather and representative invertebrate prey. Lipids were removed from each dried, homogenized sample of blood and oligochaetes using a 24 h 2:1 chloroform methanol solution rinse. This was done because lipids tend to be depleted in ^{13}C relative to whole organisms or tissues, and the lipid content of animal tissue samples can be variable, introducing bias to resulting isotope values (e.g. Peterson and Fry, 1987; Kling et al., 1992). Feather samples were rinsed using methanol solution, washed with distilled water and air dried in a fume hood.

Analysis of $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ and $\delta^{34}\text{S}$ was conducted using sub samples of approximately 0.7 mg for blood, and 0.5 mg for feather and oligochaetes. Samples were weighed into small tin cups to the nearest 0.001 mg, using a micro analytical balance. Samples were then combusted in a Eurovector (Milan, Italy) elemental analyser

(Limnological Institute, University of Constance, Germany). The resulting CO_2 and N_2 were separated by gas chromatography and passed into a Micromass (Manchester, UK) Isoprime isotope ratio mass spectrometer (IRMS) for determination of $^{13}\text{C}/^{12}\text{C}$, $^{15}\text{N}/^{14}\text{N}$ ratios. Measurements are reported in δ notation ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, respectively) where $\delta = 1000 \times (R_{\text{sample}}/R_{\text{standard}} - 1)$ ‰ relative to the Pee Dee Belemnite (PDB) for carbon and atmospheric N_2 for nitrogen in parts per thousand deviations (‰). Two sulfanilamide (Iso prime internal standards) and two Casein were used as laboratory standards for every 10 unknowns in sequence. The reference material used for sulphur isotope analysis was sulfanilamide, calibrated and traceable to NBS 127 (barium sulphate, $\delta^{34}\text{S} = +20.3$ ‰). Replicate assays of internal laboratory standards indicate measurement errors (SD) of ± 0.05 ‰, 0.15 ‰ and 0.05 ‰ for $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ and $\delta^{34}\text{S}$, respectively.

2.5. Habitat signatures

Generalized linear models with an identity link and a normal error distribution were used to evaluate whether stable isotope ratios of oligochaetes (response variable) could be explained by salinity class or hydroperiod. Model selection was based on the AIC_c criterion for small sample size (Burnham and Anderson, 2002). AIC_c is presented as weight ($\text{AIC}_c\omega$), reflecting the weight of evidence for a model being the best model for the data at hand.

2.6. Discrimination factor

Mizutani et al. (1992) estimated feather $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isotope discrimination factors for greater flamingos fed on a pellet diet as $\Delta^{13}\text{C} = 3.6 \pm 0.6$ ‰ and $\Delta^{15}\text{N} = 5.6 \pm 0.3$ ‰. However, these estimates fall outside the range of published values for other birds (e.g. Caut et al., 2009), especially with respect to $\Delta^{15}\text{N}$. Hence, we preferred to use the approach of Caut et al. (2009), estimating blood and feather discrimination factors for both carbon and nitrogen from a meta analysis incorporating the effect of diet. Accordingly, discrimination factors for feather $\delta^{13}\text{C}$ and for feather and blood $\delta^{15}\text{N}$ are independent of diet. This meant that for feather $\Delta^{13}\text{C}$ and

for feather and blood $\Delta^{15}\text{N}$, a constant discrimination factor could be applied across salinity and hydroperiod classes (Table 1; Caut et al., 2009).

For analysis of blood $\Delta^{13}\text{C}$, the equation provided by Caut et al. (2009) was applied:

$\Delta^{13}\text{C} = 0.199 \cdot \delta^{13}\text{C} + 3.289$, where $\delta^{13}\text{C}$ is the average dietary isotopic ratio for a given salinity or a given hydroperiod class (Table 1).

The approach of Florin et al. (2011) was used to estimate the sulphur discrimination factor ($\Delta^{34}\text{S}$) for blood and feather, wherein $\Delta^{34}\text{S}$ is expressed as a function of $\delta^{34}\text{S}$ along salinity and hydroperiod gradients: $\Delta^{34}\text{S} = 0.26 \cdot \delta^{34}\text{S} + 1.16$, where $\delta^{34}\text{S}$ is the average dietary isotopic ratio for a given salinity class (Table 1).

2.7. Dietary reconstitution

In order to reconstitute dietary isotopic signatures from tissue isotope signatures, it is necessary to take discrimination factors into account. The discrimination factor is formulated as:

$$\Delta X = \delta X_{\text{tissue}} - \delta X_{\text{diet}} \quad (1)$$

where δX_{tissue} and δX_{diet} are the bird tissue specific and diet specific signatures, respectively.

Following Caut et al. (2009), ΔX can be expressed as a linear function of δX_{diet} :

$$\Delta X = a + b\delta X_{\text{diet}}, \quad (2)$$

With $b = 0$ when ΔX is diet independent. δX_{diet} can then be reconstituted from Equations (1) and (2) as

$$\delta X_{\text{diet}} = \frac{\delta X_{\text{tissue}} - a}{1 - b}. \quad (3)$$

Using Equation (3), isotope values for diet were reconstituted from feather or blood signatures and compared to the actual (raw) isotopic signature of available oligochaete prey. Individual differences in tissue stable isotopes were calculated by applying paired t test and Spearman rank correlations for blood and feathers using either actual (raw) signatures or reconstituted dietary values for each isotopic element.

2.8. Isotopic shift and habitat use

We calculated an Index of Isotopic shift (I_{IS}) that measures the difference between blood and feather stable isotope ratios:

Table 1
Discrimination factors (‰) for feather and blood of greater flamingo chicks depending on salinity and hydroperiod.

	Salinity	$\Delta^{13}\text{C}$	$\Delta^{15}\text{N}$	$\Delta^{34}\text{S}$
Blood	0	1.22 ± 0.48	2.25 ± 0.20	-2.27 ± 1.02
	5	0.86 ± 0.43	2.25 ± 0.20	-2.95 ± 1.13
	10	-0.18 ± 0.75	2.25 ± 0.20	-2.99 ± 1.24
	30	-1.06 ± 0.45	2.25 ± 0.20	-1.79 ± 0.95
	40	-1.10 ± 0.45	2.25 ± 0.20	-2.53 ± 1.41
Feather	0	2.16 ± 0.35	3.84 ± 0.26	-2.27 ± 1.02
	5	2.16 ± 0.35	3.84 ± 0.26	-2.95 ± 1.13
	10	2.16 ± 0.35	3.84 ± 0.26	-2.99 ± 1.24
	30	2.16 ± 0.35	3.84 ± 0.26	-1.79 ± 0.95
	40	2.16 ± 0.35	3.84 ± 0.26	-2.53 ± 1.41
	Hydroperiod	$\Delta^{13}\text{C}$	$\Delta^{15}\text{N}$	$\Delta^{34}\text{S}$
Blood	Temporary	-0.48 ± 0.96	2.25 ± 0.20	-2.40 ± 1.05
	Semi-temporary	-0.70 ± 0.64	2.25 ± 0.20	-2.78 ± 1.03
	Permanent	-0.02 ± 1.11	2.25 ± 0.20	-2.63 ± 1.39
Feather	Temporary	2.16 ± 0.35	3.84 ± 0.26	-2.40 ± 1.05
	Semi-temporary	2.16 ± 0.35	3.84 ± 0.26	-2.78 ± 1.03
	Permanent	2.16 ± 0.35	3.84 ± 0.26	-2.63 ± 1.39

$I_{\text{IS-C}}$	$\delta^{13}\text{C}$ blood	$\delta^{13}\text{C}$ feather
$I_{\text{IS-N}}$	$\delta^{15}\text{N}$ blood	$\delta^{15}\text{N}$ feather
$I_{\text{IS-S}}$	$\delta^{34}\text{S}$ blood	$\delta^{34}\text{S}$ feather

If adults feed younger and older chicks from different dietary sources, any resulting shift in isotopic values should correlate with chick age. Since there was no *a priori* hypothesis on the exact shape of this relationship, Spearman rank correlations were used to explore whether chick age (represented by tarsus length) could explain variations in I_{IS} .

Finally, we examined whether differences in stable isotope signatures between blood and feather might be used to indicate population level variations in habitat use relative to salinity and hydroperiod using Bayesian mixing models and package SIAR of R (Parnell et al., 2008; R core team 2012). This required triple isotope ($\delta^{13}\text{C}$, $\delta^{15}\text{N}$ and $\delta^{34}\text{S}$) models with 5 endpoints for the salinity gradient and 3 hydroperiod classes, and tissue specific isotopic discrimination factors as detailed in Table 1.

2.9. Body condition and habitat use

Scaled body mass index was calculated as a proxy for flamingo chick body condition, according to Peig and Green (2009). Compared to traditional measurements of body condition calculated from the residuals of body mass and tarsus length, the scaled body mass index takes better account of the relationship between size and mass. The scaled body mass index is derived from the Thorpe Leonart model (Thorpe, 1975; Leonart et al., 2000) with a Standard Major Axis (SMA) regression:

$$\hat{M}_i = M_i [L_0 / L_i]^{b_{\text{SMA}}}$$

where M_i and L_i are the mass and tarsus length of individual i , respectively; L_0 is an arbitrary value (for instance, the arithmetic mean of tarsus length in the studied population) and b_{SMA} is the slope of SMA regression.

Generalised linear models were used to determine whether scaled body mass could be explained by the isotopic signatures of $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ or $\delta^{34}\text{S}$. Linear models were tested without interactions, with all combinations of reconstituted dietary isotopes as explanatory factors. The best model was selected based on the AIC_c criterion (Burnham and Anderson, 2002). All statistical analyses were conducted using R (R core, 2012).

3. Results

3.1. Habitat signatures

Oligochaete $\delta^{13}\text{C}$ increased with salinity (AIC_c $\omega = 0.92$ for a model retaining only this effect) but did not vary in relation to hydroperiod (AIC_c $\omega = 0.08$ for the same model adding hydroperiod; Fig. 2a and b). Oligochaete $\delta^{15}\text{N}$ increased with hydroperiod (AIC_c $\omega = 0.66$ for a model retaining only this effect) but did not vary with salinity (AIC_c $\omega = 0.34$ for a null (intercept only) model; Fig. 2c and d). Neither salinity (AIC_c $\omega = 0.02$) nor hydroperiod (AIC_c $\omega = 0.07$) appeared to have any association with oligochaete $\delta^{34}\text{S}$ (AIC_c ω of the intercept only model = 0.90; Fig. 2e and f).

3.2. Diet reconstitution and individual level feather blood isotope comparisons

Discrimination factors then allowed reconstituting diet from feather and blood raw signatures. While feather and blood isotope

Fig. 2. Variations in oligochaetes $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ and $\delta^{34}\text{S}$ signatures in relation to salinity and hydroperiod.

values differed significantly for all isotopes (Table 2), there was no significant difference in dietary values of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ reconstituted from blood and feather signatures. Reconstituted dietary values for $\delta^{34}\text{S}$ however varied significantly (Table 2). Blood and feather isotope ratios were correlated for $\delta^{13}\text{C}$ and $\delta^{34}\text{S}$ but not for $\delta^{15}\text{N}$ (Table 2).

Using reconstituted dietary values, the $\delta^{13}\text{C}$ isotopic shift for ($I_{\text{IS-C}}$) was not significantly different from zero (Table 2). However, 25% of flamingo chicks exhibited a negative $I_{\text{IS-C}}$ of more than 2‰ and 17% of these individuals showed a positive $I_{\text{IS-C}}$ greater than 2‰ ($I_{\text{IS-C}}$ range [-9.47; 6.94]). Similarly, $I_{\text{IS-N}}$ was not significantly different from zero, but 32% of individuals presented a negative $I_{\text{IS-N}}$ of more

Table 2

Actual isotope signatures of simultaneously sampled feather and blood of greater flamingo chicks and reconstituted dietary values using discrimination factors with paired differences, paired *t*-test and Spearman correlations.

	Blood	Feather	Paired difference	Paired <i>t</i> -test	Spearman correlation $\pm 95\%CI$
$\delta^{13}C$ ‰					
Actual isotope signature	-19.4 ± 3.03	-17.51 ± 3.01	-2.33 ± 3.34	<i>t</i> 3.69; df 27; $p < 0.001$	<i>r</i> 0.47 [0.07; 0.76]; <i>p</i> 0.01
Diet reconstitution	-19.25 ± 3.78	-19.67 ± 3.01	0.075 ± 3.8	<i>t</i> -0.104 ; df 27; <i>p</i> 0.99	<i>r</i> 0.47 [0.10; 0.77]; <i>p</i> 0.01
$\delta^{15}N$ ‰					
Actual isotope signature	14.11 ± 2.8	16.27 ± 2.35	-2.44 ± 3.22	<i>t</i> 3.89; df 27; $p < 0.0001$	<i>r</i> 0.10 [−0.33; 0.51]; <i>p</i> 0.60
Diet reconstitution	11.86 ± 2.8	12.43 ± 2.35	0.85 ± 3.32	<i>t</i> 1.36; df 27; <i>p</i> 0.18	<i>r</i> 0.10 [−0.32; 0.50]; <i>p</i> 0.60
$\delta^{34}S$ ‰					
Actual isotope signature	8.83 ± 3.04	11.22 ± 3.22	-3.01 ± 2.93	<i>t</i> 5.44; df 27; $p < 0.0001$	<i>r</i> 0.46 [0.08; 0.77]; <i>p</i> 0.01
Diet reconstitution	10.34 ± 4.11	13.59 ± 4.35	-4.07 ± 3.96	<i>t</i> 8.62; df 27; $p < 0.0001$	<i>r</i> 0.46 [0.08; 0.79]; <i>p</i> 0.01

than 2‰ and 14% showed a positive I_{IS-N} greater than 2‰ (I_{IS-N} range [5.80; 6.58]). In contrast, I_{IS-S} was significantly different from zero (Table 2), with 78% of individuals presenting a negative I_{IS-S} of more than 2‰ (I_{IS-S} range [12.08; 6.72]).

For all isotopic elements considered, I_S was independent of tarsus length (Spearman rank correlations: $\delta^{13}C$: r 0.05 [0.3; 0.46], p 0.76; $\delta^{15}N$: r 0.12 [0.54; 0.29], p 0.54; $\delta^{34}S$: r 0.21 [0.55; 0.17], p 0.26).

3.3. Habitat use at the population level as predicted from SIAR mixing models

At the population level, SIAR mixing models indicated that saltpans of the highest salinity class (38% [range: 16–54%]) and freshwater marshes (33% [range: 20–45%]) were the main habitats from which adult flamingos provisioned their chicks early in the breeding season (Table 3, Fig. 3a). Interestingly, reliance on salt marshes (in particular class 4 marshes with salinities of 30–40) showed a proportional increase towards the end of parental care, such that by the end of the season > 50% of chick diet originates from habitats in the two highest salinity classes (Table 3, Fig. 3b).

3.4. Habitat use and body condition of fledglings

Scaled body mass index decreased with dietary isotope values reconstituted from feather $\delta^{15}N$ only ($AIC_c\omega$ 0.36 for the model retaining only this effect, β 32.4 ± 15.4), suggesting that chicks fed from temporary marshes attained higher body condition than those fed from permanent marshes. A model adding $\delta^{13}C$ could not be discarded (ΔAIC_c 0.73; $AIC_c\omega$ 0.26) and all other models had $\Delta AIC_c > 2$. No such effect was detected using blood isotopic composition, where a model retaining no effect was preferred ($AIC_c\omega$ 1.00).

Table 3

Mean and Bayesian confidence intervals of the probability of habitat use by greater flamingos along salinity and hydroperiod gradients in the Camargue, as modeled from the Bayesian mixing model for feather and blood.

	Feather	Blood
Salinity		
0	0.33 [0.20; 0.45]	0.30 [0.13; 0.44]
5	0.04 [0.001; 0.14]	0.05 [0.001; 0.19]
10	0.14 [0.006; 0.38]	0.10 [0.003; 0.31]
30	0.08 [0.003; 0.26]	0.31 [0.05; 0.56]
40	0.38 [0.16; 0.54]	0.23 [0.01; 0.48]
Hydroperiod		
Temporary	0.06 [0.002; 0.21]	0.11 [0.005; 0.34]
Semi-temporary	0.22 [0.07; 0.36]	0.15 [0.01; 0.35]
Permanent	0.70 [0.51; 0.84]	0.73 [0.50; 0.91]

4. Discussion

Flamingos utilize a wide range of wetlands. Tracing seasonal variations in habitat use within a heterogeneous wetland complex using traditional approaches is almost impossible (but see González Solís et al., 1997; Votier et al., 2003; Takekawa et al., 2009). Several interacting factors mediate seasonal patterns of habitat use in waterbirds. The present study uses intrinsic chemical signals in blood and feather in tandem with those from in vertebrates sampled along gradients of salinity and hydroperiod to provide integrated data on resource acquisition and allocation to offspring.

For all the three elements considered in this study, actual isotope signatures differed significantly between flamingo chick blood and feather. In contrast, dietary values reconstituted from these two tissues differed significantly for $\delta^{34}S$ but not for $\delta^{13}C$ or $\delta^{15}N$. Highlighting the importance of taking into account tissue and diet specific discrimination factors, this result suggests there is no consistent shift in habitat use among greater flamingos between the early and late phases of parental care.

While these results fail to meet our prediction at the population level, presenting no consistent pattern in magnitude or direction, most of the individuals examined did indeed show a seasonal shift in isotope values. A considerable number of individuals showed either positive or negative shifts, indicating that parents do vary the habitats used to provision their offspring as the season progresses. The results also indicate a strong between individual variation in resource acquisition or allocation, but this variation is not related directly to the size of the chicks. Therefore, alternating habitat use by breeding birds is not a simple adaptive response to ontogenetic change (such as chick growth). Indeed, it is likely to be a more complex response, possibly related to individual variation in competitive ability as resources become scarce. In such circumstances, some individuals might focus on a relatively limited fraction of the available resources, while others track seasonal changes or switch to different resources. Our results support this assumption, indicating that the utilization of saline marshes increases as the season progresses. This may correspond with resource peaks known to occur in saltpans (e.g. *Artemias* spp.; Britton and Johnson, 1987) and with the drying up of freshwater and brackish temporary marshes, which typically occurs in late July in the Camargue. These findings have significant conservation implications, underlining the importance of certain seasonally available habitats at critical periods in the flamingo breeding cycle.

Our results further confirm the critical importance of saltpans for breeding greater flamingo, up to the point where chicks are fledged (44–54%; cumulating the two highest salinity classes). The constant water levels maintained in saltpans during summer make them a vital alternative to natural marshes, not only for breeding flamingos but also other coastal waterbirds such as slender billed gulls, especially in dry years (Béchet et al., 2009; Ramirez et al.,

Fig. 3. Mixing model estimates of the proportion of habitat used by greater flamingos for chick provision along the salinity gradient in early season (feather isotope signature (a)) and late season (blood isotope signature (b)) and along the hydroperiod gradient in early season (feather (c)) and late season (blood (d)). Bars represent the 50th, 75th and 95th Bayesian credibility intervals (dark grey, grey, light grey colours, respectively).

2012). In previous work we have used data from dye marked flamingos to show that use of saltpans during the hatching period increases from 18% in a normal year to 60% in a drier year (Béchet et al., 2009).

Our results suggest that flamingo chick body condition is negatively related to $\delta^{15}\text{N}$ signature, suggesting that chicks fed from temporarily flooded marshes fare better than those provisioned from more permanent ones. As temporary marshes dry out, resident invertebrates become concentrated to the point where the profitability of foraging matches or exceeds that available in local saltpans. Thus, the apparent trend for increasing use of temporary marshes (from 6% to 11%) during the breeding season could be an adaption allowing parents to maximize the body condition of their chick. As flamingo breeding numbers have been shown to correlate positively with the extent of flooding in natural marshes (Cézilly et al., 1995; Béchet and Johnson, 2008), the positive relationship between chick body condition and temporary marsh use stresses the importance of conserving this habitat. Despite their quality, temporary marshes may remain marginally used because of their

decreasing availability in the Camargue in recent decades – the price of increased availability of freshwater and permanent waterbodies (Tamisier and Grillas, 1994). Monitoring of relative habitat use by foraging flamingos suggests that while they have adjusted to this rapid environmental change, this may be at the cost of a lower chick body condition, a trend also found in slender billed gulls (Ramirez et al., 2012).

Oligochaetes, which are widespread among all habitats and known to be part of flamingo diet (Johnson and Cézilly, 2007), were used to provide values of $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ and $\delta^{34}\text{S}$ representing the source of nutrients for feather and blood cell synthesis in chicks. Processing a mixture of prey items independent of relative availability may have produced a wide range of isotope values and made interpretation of the data difficult or misleading. The isotope signatures provided by oligochaete samples fell within the range reported for other flamingo prey found in the Vaccares Lagoon, the largest Camargue wetland (Fig. 1), including copepods, *Gammarus* sp., *Cerastoderma glaucum* and *Mysis* sp. (Persic et al., 2004) and for invertebrates encountered around Fuente de Piedra, the main

flamingo breeding site in Spain (Rendón et al., 2011). Oligochaete isotope signatures also responded to salinity or hydroperiod gradients and, for the highest salinity classes, fell within the range of values reported for *Artemia* sp. in coastal salt pans ($\delta^{13}\text{C}$ from 14 to 21‰ and $\delta^{15}\text{N}$ from 1 to 12‰; Spero et al., 1993). We are thus confident that using oligochaetes alone as an index of habitat use was a reasonable approach in this study.

In summary, this study has shown that comparing dual tissue, triple stable isotope values with dietary signatures, habitat salinity and hydroperiod constitutes a useful means of tracking habitat use by breeding adult waterbirds. This isotopic approach offers a sensitive measure of highly localized environmental change, in relation to both salinity and hydrology. The same methodologies might be usefully extended to other wetland communities globally. While the Camargue salt pans are threatened with closure due to economic globalization (Béchet et al., 2012) and temporary wetlands in the region remain threatened by agriculture conversion, tourism and harbour development, our results further emphasize the critical importance of conserving permanent and seasonally available habitats for breeding birds including the greater flamingo.

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