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ORIGINAL ARTICLE

Seasonal variation in the diet of Spoonbill chicks in the Wadden Sea: a stable isotopes approach

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Abstract We used stable isotope tracers in the growing primary feathers of Eurasian Spoonbill chicks (*Platalea leucorodia leucorodia*) to study seasonal variation in their diet on one of the Frisian islands, Schiermonnikoog, The Netherlands. Using growing individual primaries as natural samplers over time, samples were taken along the length of primary feathers to estimate both within- and between-individual variation in diet. Absolute isotopic ratios of feather material ranged from −26.2 to −14.7 ‰ for carbon ($\delta^{13}C$) and from 13.0 to 18.7 ‰ for nitrogen ($\delta^{15}N$). The variation in $\delta^{13}C$ values suggests the use of a variety of feeding habitats, ranging from freshwater to marine. Across the breeding season, there was a shift from predominantly freshwater prey early on to a more marine diet later in the season. Surprisingly, this shift did not occur within the growth trajectory of early born chicks which instead showed the opposite, but it did occur within individual chicks born later in the season. Stable isotope Bayesian mixing-model (SIAR) outcomes demonstrated that the freshwater/brackish prey had the highest isotopic contribution "…(51 %; 95 % confidence interval 39-63 %) to the diet early in the breeding season, whereas marine prey contributed most (78 %; 66-89 %) to the diet later. That chicks fed with either freshwater or marine food items had similar body condition indices suggests that the eating of marine prey did not come at a major cost for growing Spoonbill chicks.

Keywords Chick body condition · Diet shift · Feather sampling · *Platalea leucorodia* · SIAR · Stable isotope analysis

Zusammenfassung

Jahreszeitliche Unterschiede in der Nahrungszusammensetzung von Löfflerküken im Wattenmeer: eine Analyse stabiler Isotope

Anhand der Signaturen stabiler Isotope in wachsenden Handschwingen von Löfflerküken (*Platalea leucorodia leucorodia*) auf Schiermonnikoog, einer der Westfriesischen Inseln (Niederlande), wurden jahreszeitliche Unterschiede in deren Nahrungszusammensetzung untersucht. Da die einzelnen wachsenden Federn praktisch natürliche Zeitreihen darstellen, nahmen wir Proben über die ganze Länge der Handschwingen, um die Variation in der Nahrungszusammensetzung sowohl bezogen auf einzelne Individuen als auch auf die Unterschiede zwischen verschiedenen Tieren abzuschätzen. Die absoluten Isotopenverhältnisse des Federmaterials lagen zwischen −26.2 ‰ und −14.7 ‰ für Kohlenstoff ($\delta^{13}C$)

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beziehungsweise zwischen 13.0 und 18.7 ‰ für Stickstoff ($\delta^{15}N$). Die Variation der $\delta^{13}C$-Werte deutet darauf hin, dass zur Nahrungssuche eine Vielzahl von Habitaten genutzt werden, die von Süßwasserhabitaten bis zu marinen Lebensräumen reichen. Im Verlauf der Brutsaison kam es zu einer Verschiebung von überwiegend im Süßwasser lebender Beute zu Beginn zu einem stärker marin geprägten Nahrungsspektrum gegen Ende der Brutzeit. Überraschenderweise ereignete sich diese Verschiebung nicht während der Wachstumsphase früh geschlüpfter Küken, bei denen stattdessen das Gegenteil zu beobachten war, sondern zeigte sich bei einzelnen Küken, welche zu einem späteren Zeitpunkt schlüpften. Die Ergebnisse der Bayes'schen Mischmodelle stabiler Isotope (SIAR) zeigten, dass Beute aus Süß- und Brackwasserhabitaten am Anfang der Brutsaison den größten Teil zur Isotopenzusammensetzung beitrug (51 %; 95 %-Konfidenzintervall: 39–63 %), wohingegen später marine Beutetiere den höchsten Anteil am Nahrungsspektrum ausmachten (78 %; 66–89 %). Dass Küken, die mit Süßwassertieren gefüttert wurden, ähnliche Konditionsindizes besaßen wie Küken, die marine Nahrung bekommen hatten, weist darauf hin, dass der Verzehr mariner Beutetiere für wachsende Löfflerküken keinen großen Nachteil darstellt.

Introduction

Optimal foraging theory predicts that animals will maximize their energy intake by seeking the most profitable prey (MacArthur and Pianka 1966). Many bird species are known to change diet in response to changes in food availability (Bryant et al. 1999; Eggers 2000; van Gils et al. 2006; Ghosh et al. 2011). However, young birds may have additional requirements and possible constraints on food selection, aspects that will change as the chicks turn into fully grown birds (Johnston and Bildstein 1990; Davoren and Burger 1999; Knoff et al. 2002; Hannam et al. 2003; Valera et al. 2005; Abraham 2008; Beaulieu et al. 2010; Steenweg et al. 2011). For example, studies have shown that a diet with higher salinity may impose physiological stress (Klaassen and Ens 1990; Johnston and Bildstein 1990, Hannam et al. 2003; Gutiérrez et al. 2011), especially affecting chicks that not yet have fully developed salt glands (Knoff et al. 2002; Hannam et al. 2003).

Although some species of birds are highly restricted in their choice of food and feeding habitats (the molluscivore Red Knot, *Calidris canutus*, can serve as an example; Piersma 2012), others are able to rely on a variety of foraging habitats to make a living. The Eurasian Spoonbill (*Platalea leucorodia leucorodia*) provides an example of a large wading bird that forages in both freshwater and marine habitats (Hancock et al. 1992). Spoonbills are tactile foragers that wade through shallow water and use bill-sweeping to catch their prey (Hancock et al. 1992; Matheu and del Hoyo 1992). They feed during both day and night (Piersma 1980; El-Hacen et al. 2013) on a wide variety of prey, mainly fish and crustaceans, but also molluscs, insects and worms (Cramp and Simmons 1977).

Based on daytime observations of foraging Eurasian Spoonbills in different estuarine habitats in the Netherlands (de Goeij et al. 1985; van Wetten and Wintermans 1986), it has been suggested that Spoonbills primarily feed in freshwater habitats at the beginning of the breeding season but gradually shift to feed in the intertidal zone (van Wetten and Wintermans 1986; Kemper 1995; Bauchau et al. 1998). This diet shift is thought to be driven by an early spring peak in the availability of anadromous Three-spined Sticklebacks (*Gasterosteus aculeatus*) in freshwater habitats (Kemper 1995; Huntingford et al. 2001; Östlund-Nilsson 2007) combined with a build-up of the availability of Brown Shrimp (*Crangon crangon*) in the nearby intertidal areas later on (Campos et al. 2010; Hufnagl and Temming 2011; Campos et al. 2012; Tulp et al. 2012). However, since the first descriptions of Spoonbill foraging in the 1980s, the Wadden Sea area has been subject to major ecosystem change (Eriksson et al. 2010), changes that may well have affected the abundance and migration strategies of Spoonbills' potential prey, including sticklebacks and Brown Shrimp.

Feathers consist of keratin that is metabolically inactive once synthesized. Keratin thus reflects the isotope values of the diet during the time when the tissue was formed (Schell et al. 1989; Mizutani et al. 1990). Carbon values of marine organisms are predictably ^{13}C enriched relative to the freshwater animals (Craig 1953; Chisholm et al. 1982; Fry et al. 1983; Fry 2002). For nitrogen, the ratio of ^{15}N to ^{14}N ($\delta^{15}N$) shows a typical progressive increase of 3.4 ‰ (on average) at each successive trophic level (Schoeninger and deNiro 1984; Hobson and Welch 1992; Minagawa and Wada 1984; Post 2002; Fry 2006). By not only sampling individuals over the course of the breeding season but by additionally sampling the material along the length of a single feather (Hobson and Clark 1992; Knoff et al. 2002), it is possible to document both inter- and intra-individual seasonal changes in the origin of diets.

Here, we verified the occurrence of a seasonal shift in the use of freshwater to marine habitats by characterizing stable isotopes (carbon and nitrogen) in the growing feathers of chicks of Spoonbills breeding on the Wadden Sea island Schiermonnikoog. We evaluated the percent contribution of several freshwater versus marine prey species in the diet. In an attempt to see whether a marine (i.e. salty) diet might compromise the growth of growing chicks (Knoff et al. 2002; Hannam et al. 2003), we assessed whether the proportion of marine food in the diet was correlated with body condition.

Methods

Feather collection and processing

We studied the Spoonbills breeding on the saltmarshes of Schiermonnikoog, the Netherlands (53°29′N, 6°15′E) during April–June 2010. Colonies were observed from a mobile hide to assess hatching dates. Within 2 weeks after hatching, chicks received individually labelled cotton bands and head-bill length was measured to accurately estimate age (Lok et al., in review). When 3–5 weeks old, 40 of these chicks were recaptured and the 5th primary feather of the left wing was collected. In addition, we collected the 5th primary feather of six chicks that were not labelled at a younger age; their age was estimated (somewhat less accurately; Lok et al., in review) from the head–bill length (HB) at 4–5 weeks old. Age was estimated from HB using the reversed Gompertz curve: age $(t) = -\ln\{-\ln[\mathrm{HB}(t)/\mathrm{HB}_{\max}]\}/k + T_{\mathrm{i}}$. Following Lok et al. (in review), we used $\mathrm{HB}_{\max} = 179$, $k = 0.053$ and $T_{\mathrm{i}} = 7.3$ for females, and $\mathrm{HB}_{\max} = 188$, $k = 0.053$ and $T_{\mathrm{i}} = 7.9$ for males. Weight (g) was measured for all chicks during feather extraction. For sex determination, a blood sample of 10–80 µl was taken from the brachial vein, stored in 96 % alcohol and analysed using molecular assays (Fridolfsson and Ellegren 1999).

Prior to subsampling, the length of the 5th primary feather was measured. Assuming that the feather started to grow immediately after hatching (though not yet visible at the outside), we estimated daily growth rate of the feather by dividing the length of the feather by the estimated age of the chick when the feather was extracted. Average daily growth rate of the feathers was 6.6 ± 0.5 mm (mean ± SD, $n = 46$). Between 3 and 5 weeks after hatching, the growth of the 5th primary feather is approximately linear (Fig. 1). Although this may not be entirely true for the first few weeks (Lok et al., in review), a linear growth of 6.6 mm/day fitted nicely through the origin and the points, with the age of the chicks estimated from their head–bill length (Fig. 1).

Feathers were cleaned with warm water and rubbed to remove dirt and external contamination, then dried in an oven at 60 °C. After drying, feather subsamples were taken along the length of the right side of the vane. The subsampling protocol was to cut a sample of 6 mm (assimilated over approximately one diurnal cycle) longitudinally every 25 mm (resulting in a time interval of approximately 4 days). The number of longitudinal subsamples of a feather was only determined by its length, varying from 2–7 subsamples per feather, resulting in a total of 203 subsamples of 46 feathers.

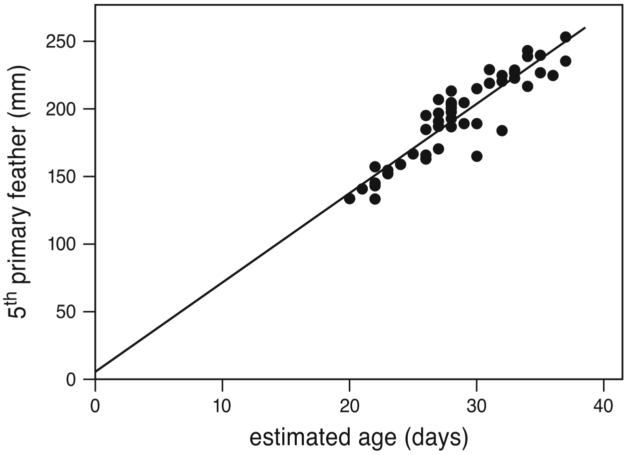

Fig. 1 The length of the 5th primary feather (in mm, measured after pulling, therefore including the entire shaft) of Eurasian Spoonbill (*Platalea leucorodia leucorodia*) chicks as a function of chick age which was estimated from head-bill length

Prey item collection and processing

In April–May 2012 and 2013, prey items were sampled with a fishing net at locations where foraging Spoonbills were regularly observed. Most prey items were collected within the foraging range of Spoonbills breeding on Schiermonnikoog, except for Gobiidae, which were only found at foraging locations elsewhere in the Wadden Sea. All collected prey items were stored frozen at −20 °C. Biometrics (total length and weight) were measured, after which the samples were freeze-dried.

Stable isotope analysis

The dried feather and prey samples were ground with a pestle and mortar. After the sample preparation was completed, we used a microbalance (Sartorius CP2P) to weigh 0.8–1 mg of the ground material into 5 × 9 mm tin capsules. Finally, capsules containing the fine fragments were analysed using a ThermoFlash 2000 elemental analyser (EA) coupled to a Thermo Delta V isotope ratio mass spectrometer. Isotope values were calibrated to an acetanilide lab standard (δ^{13}C −26.1 ‰ and δ^{15}N −1.3 ‰ calibrated on NBS-22 and IAEA-N1, respectively) and corrected for blank contribution. Stable isotope ratios were calibrated to the international standards, Vienna Pee Dee Belemnite (VPDB) for carbon and atmospheric N_2 for nitrogen. The prey samples were all analysed in duplicate and the reported values represent the mean of these two analyses. For the feather samples, a replicate was examined every tenth time to verify the precision of the isotopic measurements. The two feather samples were expected to be identical. The difference in the isotope values between a sample and its replicate ranged between 0.011 and 0.23 ‰

(with the majority less than 0.1 ‰) and reflected a measurement error of $\leq$0.2 ‰ for both δ^{13}C and δ^{15}N.

Statistical analyses

To investigate within- and between-individual changes in reliance on freshwater habitats (reflected mainly by δ^{13}C) over the breeding season, we used a mixed-effects model with random individual variation in intercept ($u_{0j} \sim N(0, \sigma_0^2)$) and slope ($u_{Wj} \sim N(0, \sigma_W^2)$). We applied the "within-subject centering" approach described by van de Pol and Wright (2009) to distinguish between within- (β_W) and between-individual (β_B) changes in δ^{13}C. In addition, we allowed for changes in within-individual patterns over the season ($\beta_{W.B}$), resulting in the following model: $y_{ij} = (\beta_0 + \mu_{0j}) + (\beta_w + \mu_{Wj})(x_{ij} - \bar{x}_J) + \beta_B(\bar{x}_J) + \beta_{W.B}(x_{ij} - \bar{x}_J)(\bar{x}_J) + \varepsilon_{0ij}$ where y_{ij} is the δ^{13}C-value of the ith feather section of individual j with x_{ij} referring to the actual assimilation date and x_j to the mean assimilation date of that individual.

We checked for, but did not find, heteroscedasticity or departures from normality in the residuals of the isotope values. Parameter values and approximate 95 % confidence intervals of the above model were estimated using the package nlme (Pinheiro and Bates 2000) in the statistical software R (v.3.0.1, R Development Core Team, Vienna, 2009). Significance of the parameters was assessed using Wald test statistics (Pinheiro and Bates 2000).

The relative contribution of several potential prey species to the diet of Spoonbill chicks was estimated using a Bayesian isotopic mixing model programmed in the R-package SIAR (v.4.0; Parnell et al. 2010). The SIAR model requires the isotopic composition of the predator (consumer) and the prey species (sources), plus the trophic enrichment factors (TEFs). Brown Shrimp, gobies (Gobiidae), perches (Percidae) and flounders (Pleuronectidae) were selected as sources, as these prey species or families are known to be common prey for Spoonbills in this area (van Dijk 1995), and contributed considerably in terms of frequency and biomass to the 7 analysed regurgitates that were occasionally produced by the chicks during ringing sessions in June–July 2011–2012 (i.e., late in the breeding season, see Appendix, Fig. A1). European Smelt (*Osmerus eperlanus*) was excluded from the analysis as this is not a typical prey for Spoonbills (Altenburg and Wymenga 1997) and was entirely absent in the regurgitates produced in 2010, the summer when the feathers were collected (Appendix, Fig. A1). Palaemonidae were included in the model as they were abundant in 6 regurgitates produced in June 2010 (Appendix, Fig. A1), were highly abundant on foraging locations of Spoonbills from Schiermonnikoog during the early season (personal observation) and have been recognized as a potentially important prey species in the literature (van Dijk 1995). Because Three-spined Sticklebacks are known to be an important prey species for Spoonbills in this area in the early season (de Goeij et al. 1985; van Wetten and Wintermans 1986; Kemper 1995), they were additionally included as a source in the model. We used the trophic enrichment factor (TEF) estimated for feathers of marine birds, being 2.16 ± 0.35 ‰ for δ^{13}C and 3.84 ± 0.26 ‰ for δ^{15}N (Caut et al. 2009). The estimated diet composition was compared between the early and late season, splitting up the feather data at 10 May, as Overdijk observed a sudden shift from observing Spoonbills foraging in freshwater to Spoonbills foraging in marine habitat around the second week of May (personal observation; also confirmed by van Dijk 1995).

To assess whether the proportion of marine food in the diet of a chick affected its body condition, we correlated the δ^{13}C value of the most recently assimilated feather section with chick body condition. As an index of body condition, we used the residual body mass divided by the predicted body mass, using the sex-specific Gompertz curves for body mass estimated by Lok et al. in review (females: $y_\infty = 1{,}485$, $k = 0.141$, $T_\mathrm{i} = 8.4$, males: $y_\infty = 1{,}741$, $k = 0.127$, $T_\mathrm{i} = 9.7$).

Results

The 46 primary feathers from Spoonbill chicks exhibited a wide range of δ^{15}N and δ^{13}C values (Fig. 2). The δ^{15}N

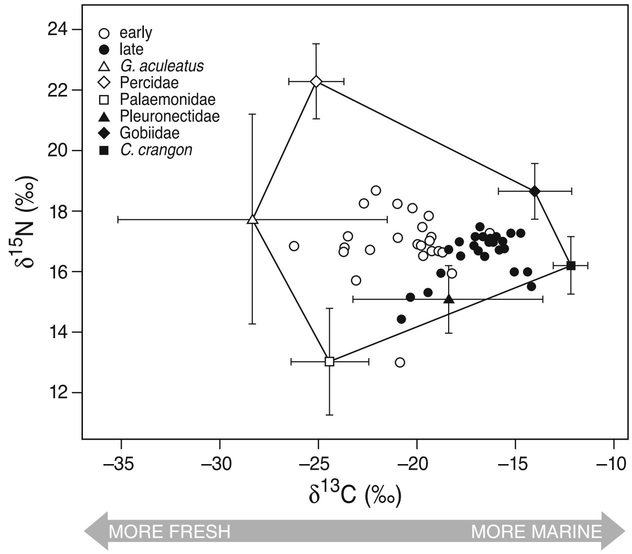

Fig. 2 δ^{13}C and δ^{15}N values of chick's feathers in the early and late season and of selected prey sources (adjusted with trophic enrichment factors): Three-spined Sticklebacks (*G. aculeatus*), Percidae, Palaemonidae, Pleuronectidae, Gobiidae and Brown Shrimp (*C. crangon*). The *minimum convex polygon* is drawn to estimate the sources of the diet of Spoonbill chicks

values ranged between 13.0 and 18.7 ‰, δ^{15}N values varied mostly within the freshwater habitat and were almost identical in the marine habitat category (Fig. 2). The δ^{13}C values ranged between −26.2 and −14.7 ‰ which indicates that the diet originated from a variety of habitats ranging from freshwater to marine sources (Bearhop et al. 1999). δ^{13}C values of feather samples differed significantly between early and late breeding stages (*t* test: $t = -7.02$, $df = 47$, $p < 0.001$).

The statistical model describing the within- and between-individual changes in δ^{13}C pattern showed that, at the population level, there was a shift towards higher δ^{13}C values across the study period, indicating an increasing reliance on marine prey (Table 1; Fig. 3). Moreover, there was a significant interaction between within-individual slopes and season. Whereas the change in δ^{13}C values within chicks born in May showed a similar pattern as that of the population, chicks born in April showed the opposite trend.

Table 1 Parameter estimates and approximate 95 % confidence intervals from the 'within-subject centering' mixed-effects model

Fixed effects	Estimate	95 % CI		*F* value	*P* value
		Lower	Upper		
β_0	−25.82	−27.86	−23.79	644.20	<0.001
β_B	0.17	0.12	0.23	47.23	<0.001
β_W	−0.31	−0.49	−0.11	11.18	0.002
β_{WB}	0.008	0.003	0.012	12.43	0.001
Random effects					
σ_0^2	2.85	1.82	4.46		
σ_W^2	0.014	0.006	0.035		
σ_{res}^2	0.68	0.51	0.91		

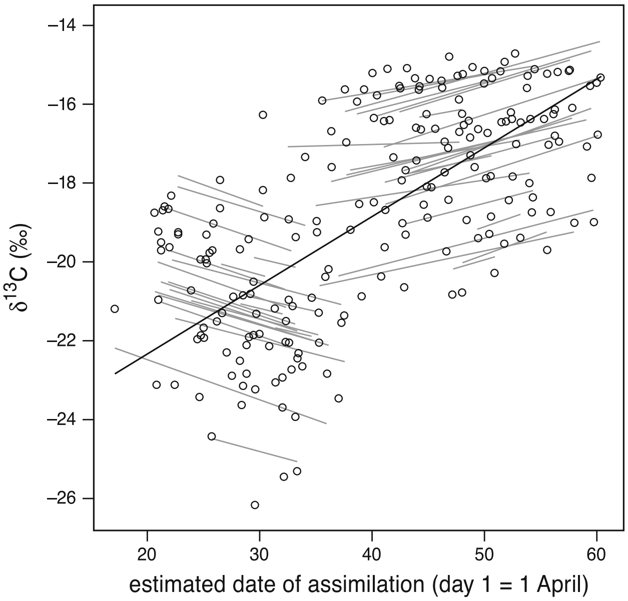

Fig. 3 δ^{13}C values as a function of the estimated assimilation date, which assumes that the feather grows linearly by 6.6 mm per day (see Fig. 1). *Open circles* represent the 203 subsamples from the 46 feathers. The *black line* shows the between-individual slope and the *grey lines* the individual slopes, estimated by the model shown in Table 1

The SIAR mixing model confirmed an increasing reliance on marine prey through the breeding season (Table 2; Fig. 4). The model estimated that the chicks received 51 % (95 % confidence interval 39–63 %) of their diet from freshwater/brackish sources (Three-spined Sticklebacks, Percidae and Palaemonidae) early in the season and 78 % (66–89 %) from marine sources (Gobiidae, Pleuronectidae and Brown Shrimp) later in the season.

We did not find a significant correlation between the body condition index at the time when the feather was collected and the proportion of marine food in the diet (reflected by the δ^{13}C value of the most recently assimilated feather section; $F_{1,44} = 0.56$, $P = 0.46$).

Discussion

We analysed stable isotopes in the feather material of chicks to investigate changes in the reliance of Spoonbills on freshwater versus marine prey for feeding. It has been reported that the use of only a small section of the feather or the homogenisation of the entire vane leads to great loss of spatiotemporal information about the diet during the feather growth (Hobson and Clark 1992; Knoff et al. 2002; Wiley et al. 2010). Taking multiple samples from the same feather is becoming more common in the isotopic studies on the feeding biology of birds (Thompson and Furness 1995; Knoff et al. 2002; Wassenaar and Hobson 2006; Smith et al. 2008; Wiley et al. 2010, 2012; Quillfeldt et al. 2010; Jaeger et al. 2010; Jensen et al. 2012). However, to our best knowledge, this is the first time that the longitudinal sampling along a feather with known growth rates is used to track the temporal variation in the diet of individual Spoonbill chicks. In this way we could test whether a population-level change in diet over the breeding season was caused by within- and/or between-individual changes. We have shown that within-individual trends did not follow the overall trend line early in the breeding season (Fig. 3).

We found a spatio-temporal diet shift in Spoonbill chicks fed with freshwater/brackish prey early in the breeding season towards more marine prey later on (Table 1; Figs. 3, 4). This shift mirrors the observation of increasing numbers of Spoonbills foraging in the Wadden Sea through the breeding season made in earlier years (de Goeij et al. 1985; van Wetten and Wintermans 1986; Kemper 1995; van Dijk 1995; Bauchau et al. 1998). The

Table 2 Summary of the isotopic values of the sources (mean ± SD) and the estimated percent contribution of the different sources to the diet of Eurasian Spoonbill (*Platalea leucorodia leucorodia*) chicks, with 95 % confidence intervals (CI)

Source (habitat)	*n*	δ^{13}C	δ^{15}N	Percent contribution			
				Early		Late	
				Mean	95 % CI	Mean	95 % CI
G. aculeatus (F)	5	−30.50 ± 6.51	13.90 ± 3.21	0.21	0.04–0.35	0.07	0.00–0.16
Percidae (F)	12	−27.23 ± 1.06	18.45 ± 0.98	0.13	0.01–0.25	0.05	0.00–0.11
Palaemonidae (F/B)	15	−26.56 ± 1.65	9.19 ± 1.50	0.17	0.03–0.31	0.10	0.00–0.21
Pleuronectidae (M)	12	−20.52 ± 4.48	11.26 ± 0.86	0.18	0.00–0.34	0.22	0.01–0.40
Gobiidae (M)	19	−16.14 ± 1.51	14.83 ± 0.65	0.15	0.00–0.30	0.21	0.01–0.40
C. crangon (M)	10	−14.32 ± 0.54	12.38 ± 0.69	0.16	0.00–0.30	0.35	0.13–0.58

Habitat denotes where the prey was caught: freshwater (*F*), brackish (*B*) or marine (*M*)

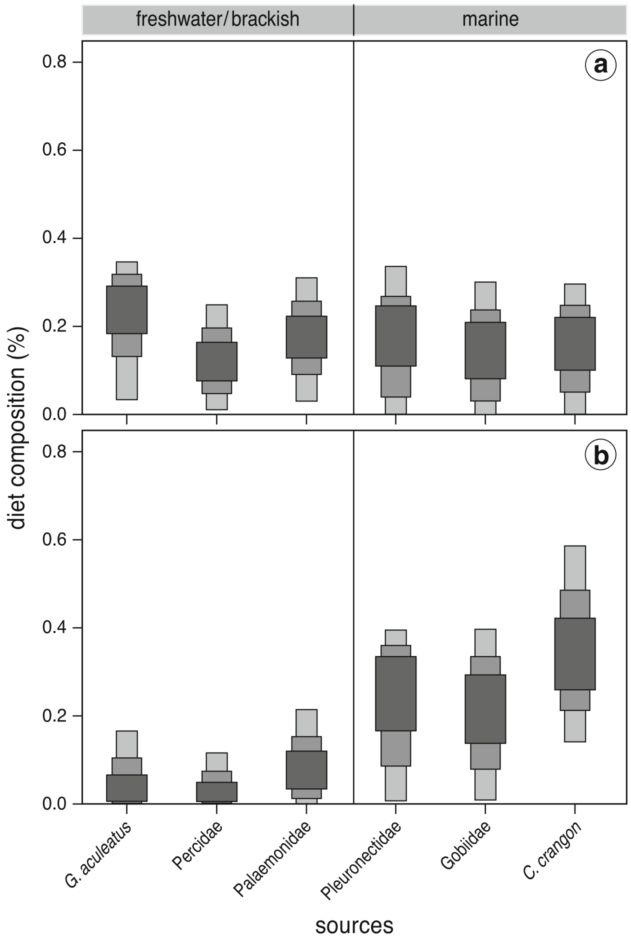

Fig. 4 Bayesian mixing model (SIAR) results showing the estimated diet composition (95, 75 and 25 % credible intervals) of Spoonbill chicks in the **a** 'early' feather samples (assimilated before 10 May) and **b** 'late' feather samples (assimilated after 10 May)

SIAR mixing model demonstrated that Spoonbill chicks were fed predominantly with freshwater/brackish prey early in the season and mostly marine prey later in the season (Table 2; Fig. 4). In early spring—from March until May—Brown Shrimp are not yet available in the shallower areas of the Wadden Sea (Beukema 1992). In the meantime, freshwater prey such as sticklebacks may be suitable prey (de Goeij et al. 1985; van Wetten and Wintermans 1986; Altenburg and Wymenga 1997). In the study area, two types of non-migratory sticklebacks, Ten-spined Sticklebacks (*Pungitius pungitius*) and Three-spined Sticklebacks, and the anadromous migratory Three-spined Sticklebacks concentrate in the freshwater ditches early in spring to reproduce (de Goeij et al. 1985; Altenburg and Wymenga 1997). Later in spring, after spawning, the adult anadromous sticklebacks die and their numbers decrease dramatically in freshwater bodies (Kemper 1995). Moreover, growing vegetation closes the ditches in such a way that Spoonbills may not be able to forage there anymore and are forced to seek food elsewhere. While Spoonbills could still forage in nearby open freshwater habitats on prey species such as Common Roach (*Rutilus rutilus*), Common Rudd (*Scardinius erythrophthalmus*), European Perch (*Perca fluviatilis*), Common Carp (*Cyprinus carpio*) and Bream (*Abramis* spp.) (Altenburg and Wymenga 1997), Brown Shrimp are meanwhile becoming available in high numbers in the surrounding intertidal areas in the Wadden Sea (Campos et al. 2010, 2012; Hufnagl and Temming 2011; Tulp et al. 2012).

Intriguingly, the seasonal trend towards higher δ^{13}C values as the breeding season progressed was shown by the chicks born later in the season, but not by the chicks born early (Fig. 3). As anadromous sticklebacks move from marine to freshwater in early spring, which will make their isotopic values to gradually change from a marine to a freshwater signal (Bearhop et al. 2002), the apparent increase in the proportion of freshwater prey in the Spoonbill chick diet in the early season may reflect the changes over time in isotopic values of the consumed migratory sticklebacks. Alternatively, Spoonbills may gradually include more true freshwater prey in their diet, because the anadromous sticklebacks, that probably have an intermediate freshwater-marine isotope values, may become less available over time as suggested above.

In some birds, young chicks suffer from a salty diet (Harriman 1967; Barnes and Nudds 1991; Dosch 1997; Knoff et al. 2002; Hannam et al. 2003). Spoonbill chicks fed mainly marine prey were not in poorer condition than chicks fed freshwater prey. As sticklebacks and Brown Shrimp are of similar (relatively low) nutritive value (Massias and Becker 1990), this suggests that a high proportion of marine food (a salty diet) did not come with major physiological costs for Spoonbill chicks of the age of 3–5 weeks old. This also helps explain why Mauritanian Spoonbills (*P. l. balsaci*) are able to breed in an entirely marine environment (the Banc d'Arguin, Mauritania; see Piersma et al. 2012; El-Hacen et al. 2013) with no freshwater available.

There was hardly any seasonal overlap between the early chicks that had a low and generally decreasing $\delta^{13}C$ signal and the later chicks that on average had a higher and slightly increasing $\delta^{13}C$ signal (Fig. 3). Rather than being caused by an arbitrary selection of chicks for this study, there were in fact no chicks raised in the intermediate period. If this gap turned out to be a systematic feature of the timing of breeding of Spoonbills in the Wadden Sea, this would indicate some degree of individual food specialization among breeding birds related to breeding time. Assuming that freshwater food (mainly sticklebacks) is more abundant early in the season, in contrast to marine food resources (mainly shrimp) that become more abundant later on, freshwater habitat specialists are expected to breed earlier than marine habitat specialists. This hypothesis now requires testing by observations in multiple breeding seasons, and the tracking of individual Spoonbills breeding early and late to investigate their foraging habitat usage in relation to diet and the availability of freshwater and marine food resources.

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