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The diet of great tit nestlings: Comparing observation records and stable isotope analyses

Emilio Pagani-Núñez^{a,b,*}, María Renom^a, Fernando Mateos-Gonzalez^{a,c,d},
Javier Cotín^e, Juan Carlos Senar^a

^aEvolutionary and Behavioural Ecology Research Unit, Natural History Museum of Barcelona, Psg. Picasso s/n., 08003 Barcelona, Spain

^bBehavioral and Community Ecology, Conservation Biology Group, College of Forestry, Guangxi University, No. 100 Daxue Road, Nanning, Guangxi 530005, People's Republic of China

^cDepartment of Integrative Biology, University of Texas, Austin, USA

^dDepartment of Collective Behaviour, Max Planck Institute for Ornithology, Germany

^eDepartment of Animal Biology, University of Barcelona, Av. Diagonal 643, 08028 Barcelona, Spain

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Abstract

The diet of wild animals has been studied using many different strategies, approaches and methods in recent decades. In this regard, stable isotopes analysis (SIA) is becoming a widespread tool, but no study has yet, to our knowledge, compared diet estimations from SIA with direct observations of the diet of passerine nestlings. Accordingly, our aim was to test the predictive power of SIA for this purpose and identify potential confounding factors such as habitat effects. To do this, we compared isotopic signatures of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ in the feathers of great tit (*Parus major*) nestlings, and the corresponding estimates of their diet based on stable isotope mixing models, with prey proportions delivered by their parents obtained through video-recordings. Between-nest differences in isotopic signatures of $\delta^{15}\text{N}$ were larger than within-nest differences. We found that $\delta^{15}\text{N}$ signatures of nestling feathers correlated positively with the proportion of spiders and negatively with the proportion of caterpillars in the nestlings' diet, the most important prey types. On the other hand, between-nest and within-nest differences in $\delta^{13}\text{C}$ ratios were of similar magnitude and $\delta^{13}\text{C}$ ratios correlated mainly with the proportion of trees surrounding nest-boxes that were *Quercus* spp. Estimates of diet composition based on mixing models correlated with the observed nestling diet, yet effect sizes were quite low. Although mixing models are commonly used to ascertain diets, our data show that they can provide valuable information on the relative intake of prey types from different trophic levels; but when complex dietary patterns are recorded (e.g. due to the confounding effects of habitat and/or temporal variation) it can be difficult to draw firm conclusions about diet composition.

Zusammenfassung

Die Nahrung von Wildtieren wurde in den letzten Dekaden mit vielfältigen Strategien und Methoden untersucht. Die Analyse von stabilen Isotopen (SI) wird immer häufiger angewandt, aber bis jetzt hat noch keine Untersuchung –soweit uns bekannt– SI-Analysen mit direkten Beobachtungen der Nahrung von Singvogelnestlingen verglichen. Unser Ziel war es, die Vorhersagefähigkeit von SI-Analysen zu prüfen und das Potential von Störfaktoren (z.B. Habitateffekte) zu bestimmen.

*Corresponding author at: Evolutionary and Behavioural Ecology Research Unit, Natural History Museum of Barcelona, Psg. Picasso s/n., 08003 Barcelona, Spain. Fax: +34933101999.
E-mail address: emipanu@outlook.com (E. Pagani-Núñez).

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Wir verglichen die $\delta^{13}\text{C}$ - und $\delta^{15}\text{N}$ -Signaturen in den Federn von Nestlingen der Kohlmeise (*Parus major*) und die entsprechenden mit Isotop-Mischungsmodellen geschätzte Zusammensetzung ihrer Nahrung mit den Anteilen der Beutearten, die von den Eltern verfüttert wurden (Auswertung von Videoaufzeichnungen).

Die Unterschiede bei den $\delta^{15}\text{N}$ -Signaturen waren zwischen den Nestern größer als innerhalb der Nester. Die $\delta^{15}\text{N}$ -Signaturen der Nestlingfedern korrelierten positiv mit dem Anteil der Spinnen und negativ mit dem der Raupen in der verfütterten Nahrung. Spinnen und Raupen waren die wichtigsten Beutearten. Andererseits waren die Unterschiede hinsichtlich der $\delta^{13}\text{C}$ -Verhältnisse zwischen und innerhalb der Nester von gleicher Größenordnung, und die $\delta^{13}\text{C}$ -Signaturen korrelierten hauptsächlich mit dem Anteil der Eichenbäume (*Quercus* spp.) in der näheren Umgebung der Nistkästen. Die mit dem Mischungsmodell geschätzte Zusammensetzung der Nahrung korrelierte mit der beobachteten Zusammensetzung, aber die Effektstärke war recht gering. Obwohl Mischungsmodelle weithin genutzt werden, um Diäten zu bestimmen, zeigen unsere Daten, dass sie wertvolle Informationen zur relativen Aufnahme von Beutearten aus unterschiedlichen trophischen Ebenen liefern können. Wenn aber komplexe Nahrungsmuster festgestellt werden (z.B. durch störende Einflüsse von Habitat und/oder zeitlicher Variation), kann es schwierig werden, sichere Schlüsse hinsichtlich der Nahrungszusammensetzung zu ziehen.

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Keywords: Great tit; Nestling diet; *Parus major*; Passerines; Stable isotopes

Introduction

Offspring diet shows great inter- and intra-population variability, which has been related to many different factors such as different personality types (Arnold, Ramsay, Donaldson, & Adam 2007; David, Cézilly, & Giraldeau 2011), parental ornamentation (García-Navas, Ferrer, & Sanz 2012; Pagani-Núñez & Senar 2014), habitat structure (Rytkonen & Krams 2003; Tremblay, Thomas, Blondel, Perret, & Lambrechts 2005), and offspring fitness (Eeva, Sillanpää, & Salminen 2009). As a consequence, the study of offspring diet has been the focus of intense research and debate in the field of ecology for many decades (see Stephens, Brown, & Ydenberg 2007 for a review).

Any study on offspring diet relies on the assumption that the sampling method accurately describes the food provided by parents. Although direct observation could be considered the most reliable approach to study animal diet, it is subject to limitations such as the lack of sensitivity to detect temporal changes or the introduction of biases related to prey size or conspicuousness (Currie, Nour, & Adriaensen 1996; Margalida, Bertran, & Boudet 2005). It is also a time-consuming approach. Conventional methods of diet analysis, such as the study of pellets, faeces or neck collars are also constrained by several biases. For instance, neck collars will usually lead to the underestimation of small prey items, while faeces are limited by differential digestion of different prey types (Poulsen & Aebischer 1995; Moreby & Stoate 2000). To evaluate their accuracy, much research has focused on comparing techniques and alternative methods (Redpath, Clarke, Madders, & Thirgood 2001; Votier, Bearhop, MacCormick, Ratcliffe, & Furness 2003). These traditional approaches have progressively been combined with (Ramos, Ramírez, Sanpera, Jover, & Ruiz 2009; Resano, Hernández-Matías, Real, & Parés 2011) and sometimes substituted by (Moreno, Jover, Munilla, Velando, & Sanpera 2010; Vitz & Rodewald

2012) a laboratory technique: stable isotopes analysis (SIA). The analysis of isotopic signatures from animal tissues provides reliable data about diet, and many studies have been conducted on the topic in the last twenty years (Inger & Bearhop 2008; Boecklen, Yarnes, Cook, & James 2011). This technique has several advantages. For instance, it is possible to collect many samples and analyse them in the laboratory *a posteriori*, and sample preparation and analyses are quite fast. In relation to terrestrial trophic ecology, $\delta^{13}\text{C}$ ($^{13}\text{C}/^{12}\text{C}$) is mainly used to infer differences in the exploitation of different plant types (C_3 and C_4), although their values have also been used as a proxy of individual diet specialisation (Hobson 1999; Kelly 2000; Araújo, Bolnick, Machado, Giaretta, & dos Reis 2007). $\delta^{15}\text{N}$ ($^{15}\text{N}/^{14}\text{N}$) is used to estimate the trophic position in the food web (Kelly 2000; Post 2002; Boecklen et al. 2011). Bayesian mixing-models are the most common approach to ascertain animal diets on the basis of isotopic signatures (e.g. SIAR, Parnell, Inger, Bearhop, & Jackson 2010). These models make possible to estimate diets using isotopic signatures of consumers and food resources. However, in many cases this technique has been used without proper field testing. Indeed, no study has yet compared direct observations and stable isotope ratios of nestling diet in passerines (but see e.g. Robb et al. 2011; Beaulieu & Sockman 2012; Cross, Hentati-Sundberg, Österblom, McGill, & Furness 2014 for studies using this technique to ascertain passerines' diet).

Great tits (*Parus major* (Linnaeus 1758)) are especially suited and attractive to address this issue. This colourful passerine is broadly distributed throughout Europe and Asia (Gosler, 1993). Mediterranean great tits are an excellent model because parents feed their nestlings with highly variable diets consisting of prey from different trophic levels (Pagani-Núñez, Valls, & Senar 2015). In our area, pines (*Pinus* spp.) and oaks (*Quercus* spp.) co-occur in different proportions along a steep gradient of variation, a pattern which is reflected in diet composition (Pagani-Núñez, Uribe,

Hernández-Gómez, Muñoz, & Senar 2014), and allows to test for habitat structure effects. We also note that, in contrast to larger birds, passerines collect food for their nestlings in a small area surrounding their nests (Royama 1966; Krebs 1971), which allows an easier and more meaningful test of the effect of habitat structure on diet.

The aim of this work was to compare the proportions of the most important prey types recorded through direct observation and isotopic ratios of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of nestling feathers. Since the most common prey types of great tits are caterpillars and spiders, which belong to different trophic levels and, hence, should show different isotopic signatures, it could be possible to assess the relative intake of these prey types using $\delta^{15}\text{N}$ values (Kelly 2000; Post 2002). Since $\delta^{13}\text{C}$ is mainly used to infer differences in the exploitation of different plant types, we also tested whether $\delta^{13}\text{C}$ was influenced by habitat structure (Hobson 1999; Kelly 2000), or whether it mainly responded to a site-specific pattern (Charmantier, Blondel, Perret, & Harmelin-Vivien 2014). We also compared estimates of nestling diet based on Bayesian mixing-models with direct observations of parental feeding behaviour. Bayesian mixing-models are becoming a widespread tool, though there is a lack of studies directly contrasting these estimations with observed diets, and potential biases (e.g. habitat and site-related effects, which can be very relevant in small passerines) should be identified.

Materials and methods

Fieldwork

We carried out our study during the spring of 2012 in the Can Catà field station (see Pagani-Núñez et al. (2014) for more details of the area), in Barcelona (NE Spain). We checked a total of 182 nest boxes twice a week to determine nest building state, laying date, hatching date, and brood size. We trapped chicks inside the nest-box at the age of 16 days (Quesada & Senar 2007). Mean brood size (\pm S.D.) was 4.7 ± 1.4 , a low record compared to previous years (Pagani-Núñez, Ruiz, Quesada, Negro, & Senar 2011; Pagani-Núñez & Senar 2014). We collected the fifth tail feather (right side, rectrix 5) of 143 nestlings from 37 nests. We collected this feather because tail feathers grow throughout the whole nestling period. We excluded from SIA two nestlings from each to ten nests (twenty individuals), which were randomly selected, as these nestlings were used in a cross-fostering experiment.

During 2008 and 2014 we also sampled $N=37$ caterpillars and $N=66$ spiders, the most important prey items in this population (Pagani-Núñez et al. 2011; Pagani-Núñez & Senar 2014). We collected these samples in the same area where our nest-boxes were located and at the same time that great tits were breeding (we carried out a monthly sampling session from April to June). We searched for arthropods beating branches of trees and bushes over a plastic sheet. Prey

specimens were stored in micro-tubes. We stored feathers in dark, dry conditions. We rapidly transported and froze arthropods at -80°C until use in the lab.

In order to measure habitat structure we counted the number of oaks (*Quercus cerrroides* and *Quercus ilex*) and pines (*Pinus halepensis*) within a radius of 25 m around the nest-boxes. We then computed the proportion of oaks as a proxy of habitat structure (Pagani-Núñez & Senar 2014; see also Pagani-Núñez et al. 2014 for a detailed description of habitat structure in our study area). To compute the proportion of trees surrounding nest boxes that were *Quercus* spp. we counted the number of trees with a trunk diameter higher than 5 cm in a radius of 25 m and determined how many of them were *Quercus* spp.

Video-recordings

We recorded nestling diet using Micro-D cameras (Mini Colour Sony IR Camera SK-C170IR) attached to the nest-box cover and focused on the entrance (see Pagani-Núñez and Senar 2014 for more details). These cameras are provided with an infrared view and a motion sensor. We filmed the chicks when they were 10–14 days old, in the period of their highest intake of food (Naef-Daenzer & Keller 1999). To minimize the possible effects produced by the installation of the camera, we camouflaged this with branches and vegetation. We installed and activated cameras at 13 h on the first day. Parents readily continued their usual behaviour after cameras were installed.

To carry out the comparison with isotopic signatures of feathers we used the recordings obtained during the first five hours of the second day (from 7 a.m. to 12 a.m., excluding the period from dawn to 7 a.m.). In this population, prey proportions delivered to nestlings by parents are highly consistent through time (Pagani-Núñez & Senar 2013), so this time period can be considered a good approximation to nestling diet. We set each feeding action in which caterpillars or spiders were observed to record prey type and exact time. We could not observe the assignment of each prey item to each nestling, and therefore pooled all the information from each nest. We determined the size of each prey item accordingly to a semi-quantitative scale in relation to beak size: 1 = small (smaller than beak size), 2 = medium (similar to beak size), 3 = large (larger than beak size) (Barba, López, & Gil-Delgado 1996). We calculated arithmetic mean values (\pm SD) of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ ratios and computed the number and mean size of spiders and caterpillars per nest. We standardised prey proportions by prey size multiplying the number of prey items of each type by their mean size and calculated new proportions on this basis (as in Pagani-Núñez et al. 2015). We focused on these two prey types because they represent the most relevant prey types for the diet of the Great tit (Naef-Daenzer & Keller 1999; Naef-Daenzer, Naef-Daenzer, & Nager 2000).

Stable isotopes

We performed a SIA of caterpillars and spiders, and of nestling tail feathers. We cleaned nestling feathers in a solution of NaOH (0.25 M) and oven-dried at 40 °C for 24 h. We washed off lipids from arthropods using chloroform-methanol (two parts of chloroform by one of methanol). We later oven dried arthropods at 40 °C during 24 h (Sweeting, Polunin, & Jennings 2006). However, we did not extract lipids from feathers since they have very low lipid content and this procedure does not affect their isotopic signatures (Mizutani, Fukuda, & Kabaya 1992). To homogenise samples, we froze arthropods contained inside micro-tubes with liquid nitrogen, which has no effect on isotopic values (Michener & Lajtha 2007), and pounded to convert them into an extremely fine powder using an agate mortar. To analyse feathers, we used the tip of the proximal part of the rachis, because it is the last part to grow and theoretically comprises prey proportions recorded in the middle stage of nestling development (see e.g. Cherel, Jaquemet, Maglio, & Jaeger 2014). Moreover, we consistently sampled all nestling feathers in the same stage of growth. We were unable to use conventional methods of homogenisation (see e.g. Resano et al. 2011) given the small size of our samples. We loaded subsamples of 0.35 mg (for both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) in tin recipients and crimped for combustion. We conducted isotopic analysis using an elemental analysis-isotope ratio mass spectrometry (EA-IRMS) with a Flash 1112 (for C and N) elemental analyser coupled to a Delta C isotope ratio mass spectrometer via a CONFLOIII interface (Thermo Fisher Scientific, Bremen, Germany). The laboratory work was carried out at the Scientific Technical Services Department at the University of Barcelona. We expressed stable isotope ratios as parts per thousand (‰), according to the following equation:

$$\delta X = [(R_{\text{sample}}/R_{\text{standard}}) - 1],$$

where X is ^{13}C or ^{15}N and R is the corresponding ratio $^{13}\text{C}/^{12}\text{C}$ or $^{15}\text{N}/^{14}\text{N}$. We referenced samples against international standards: Pee Dee Belemnite (VPDB) for ^{13}C , atmospheric nitrogen (AIR) for ^{15}N . Additionally, we used IAEAACH7, USGS40 and IAEAACH6 as internal standards for ^{13}C , and USGS40, IAEAN1, IAEANO3 and IAEAN2 as internal standards for ^{15}N . We paired internal standards against international standards and analysed after every 16 sample measurements. Standard deviation (SD) of the internal standards was ± 0.12 . The measurement precisions for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ were 0.15‰ and 0.25‰, respectively.

We analysed tail feathers in 2012. We collected and analysed prey items (caterpillars and spiders) in 2008 and 2014. In order to test whether the yearly mismatch between the collection of feathers and of prey could affect results, we performed a factorial analysis of variance with $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ ratios as dependent variables, and year (2008 or 2014), prey type (caterpillars or spiders) and its interaction as categorical factors. We computed the mean isotopic signatures of the

Table 1. Sample size, mean, minimum, maximum and standard deviation of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of great tit nestling feathers and their most common prey types, caterpillars and spiders.

	N	Mean (‰)	Min (‰)	Max (‰)	S.D.
Great tit nestlings					
$\delta^{13}\text{C}$	143	-24.84	-26.23	-22.96	0.56
$\delta^{15}\text{N}$	143	+0.27	-2.19	+3.71	1.30
Spiders					
$\delta^{13}\text{C}$	66	-25.27	-27.84	-23.18	1.13
$\delta^{15}\text{N}$	66	-0.61	-4.73	+3.44	1.58
Caterpillars					
$\delta^{13}\text{C}$	37	-26.09	-29.06	-22.22	1.62
$\delta^{15}\text{N}$	37	-4.53	-9.53	-0.07	2.16

two years for each prey type to account for possible yearly differences, carrying out in this way a conservative analysis (see Table 1 for descriptive statistics).

Statistical analyses

First, we provide information on prey proportions (Mean \pm SD). Second, we analysed variability between years in isotopic signatures of prey. We ran a general linear model with prey isotopic signatures (of caterpillars and spiders) as dependent variable, and year, prey type and its interaction as factors.

We assessed the level of variance in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ ratios among and within nests through an analysis of repeatability. Repeatability (the intra-class correlation coefficient) is the proportion of variation that can be attributed to between-group differences (Nakagawa & Schielzeth 2010), in our study between nests. Here we used this statistic as a way of testing whether isotopic ratios were more variable between than within nests. To perform this analysis we randomly selected four individuals per nest from a sub-sample of twenty-four nests, which correspond to the nests that had four nestlings or more.

We then computed a multiple linear regression to investigate the correlation between $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ ratios and prey proportions (caterpillars and spiders) and habitat structure. In the first model, mean $\delta^{13}\text{C}$ ratio per nest was our dependent variable, with the proportion of caterpillars and the proportion of oak trees around the nest-boxes as independent variables. In the second model mean $\delta^{13}\text{C}$ ratio per nest was again our dependent variable, with the proportion of spiders and the proportion of oak trees around the nest-boxes as independent variables. We did the same using mean $\delta^{15}\text{N}$ ratio per nest as dependent variable. We checked and accomplished linear model assumptions for linearity and additivity (through an examination of residual vs. predicted values) and for normality of the error distribution (residuals were normally distributed). For these analyses we used Statistica 6.0 (StatSoft 2001).

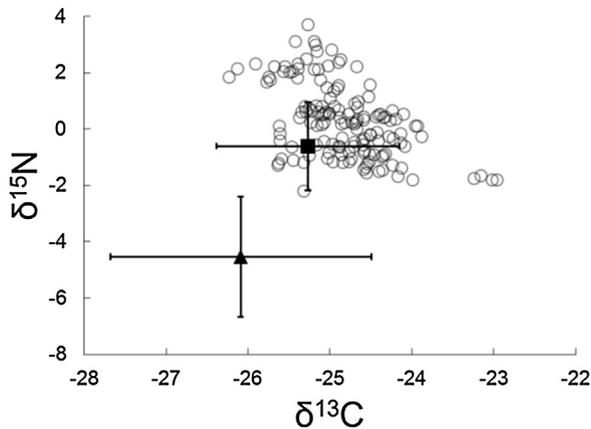


Fig. 1. Relationship between $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ signatures of *Parus major* nestlings (open circles; $N = 143$) and those of two prey types (means with SD bars): spiders (square; $N = 66$) and caterpillars (triangle; $N = 37$).

Finally, we used the package MixSIAR GUI v 2.1 (<https://github.com/brianstock/MixSIAR>; Stock & Semmens 2013) of R v 3.0.2, (<http://www.r-project.org>; R Core Development Team 2014) to estimate prey proportions using isotopic values from feathers and prey items (Fig. 1). For these analyses we focused on the estimated proportion of caterpillars and spiders ($N = 37$ nests). We had to estimate adequate Trophic Enrichment Factors (TEF) based on bibliography (model species: garden warbler *Sylvia borin*; Hobson & Bairlein 2003). These factors describe how isotopic signatures change from the food to the tissue of the consumer. We used $+3.2\text{‰}$ for $\Delta^{15}\text{N}$ and $+1.9\text{‰}$ for $\Delta^{13}\text{C}$, for both caterpillars and spiders. Different TEF's described in the literature produced less accurate results (see a discussion of this issue below). We used residual * process error structure. Nest was fixed as random factor and the proportion of oak trees around the nest-boxes as continuous effect. We run the model two times ("very long" option) to see if we obtained a consistent result. We tested the prediction that dietary estimates based on SIA were in concordance with estimates based on visual observations.

Results

Recordings showed that caterpillars were the main prey fed to nestlings by their parents (Mean \pm SD = 0.52 ± 0.22), followed by spiders (Mean \pm SD = 0.13 ± 0.08). Remaining prey items were below ten per cent, and consisted of a great variety of arthropods, in addition to fruits. Unidentifiable prey represented (Mean \pm SD) 0.07 ± 0.05 of the total.

We found that $\delta^{15}\text{N}$ ratios differed between prey types ($t = 9.75$, $P < 0.01$; Table 1, Fig. 1) but not between years ($t = -1.42$, $P = 0.16$; see Appendix A: Table 1 in Supplementary data) and the interaction between prey type and year was not significant ($t = -1.00$, $P = 0.32$). Conversely, $\delta^{13}\text{C}$

Table 2. Multiple linear regression describing the relationships between: (1) $\delta^{13}\text{C}$ of nestling feathers, our dependent variable, and the proportion of oak trees around the nest-boxes and the proportion of caterpillars delivered by parents to their nestlings; (2) $\delta^{13}\text{C}$ of nestling feathers and the proportion of oak trees around the nest-boxes and the proportion of spiders delivered by parents to their nestlings; (3) $\delta^{15}\text{N}$ of nestling feathers and the proportion of oak trees around the nest-boxes and the proportion of caterpillars delivered by parents to their nestlings; and (4) $\delta^{15}\text{N}$ of nestling feathers and the proportion of oak trees around the nest-boxes and the proportion of spiders delivered by parents to their nestlings. Significance level was $P < 0.05$.

(1) $\delta^{13}\text{C}$

	$F_{1,34}$	β	P
Intercept	12,064.53		<0.01
% <i>Quercus</i> spp.	14.28	-0.54	<0.01
% Caterpillars	0.10	-0.05	0.76

(2) $\delta^{13}\text{C}$

	$F_{1,34}$	β	P
Intercept	18,694.30		<0.01
% <i>Quercus</i> spp.	13.44	-0.53	<0.01
% Spiders	0.56	-0.11	0.46

(3) $\delta^{15}\text{N}$

	$F_{1,34}$	β	P
Intercept	0.83		0.37
% <i>Quercus</i> spp.	2.27	0.23	0.14
% Caterpillars	5.28	-0.36	0.03

(4) $\delta^{15}\text{N}$

	$F_{1,34}$	β	P
Intercept	4.67		0.04
% <i>Quercus</i> spp.	1.38	0.18	0.25
% Spiders	7.38	0.41	0.01

ratios did not show significant differences between prey types ($t = 1.68$, $P = 0.10$, Table 1, Fig. 1), while showed almost significant differences between years ($t = 1.94$, $P = 0.06$; see Appendix A: Table 1 in Supplementary data) and a different pattern between years and species was apparent ($t = -2.39$, $P < 0.01$).

Repeatability scores comparing the diet of the nestlings within a nest were higher for $\delta^{15}\text{N}$ ($r_1 = 0.91$; $P < 0.01$) than for $\delta^{13}\text{C}$ ($r_1 = 0.43$; $P < 0.01$). That is, differences between nests in $\delta^{15}\text{N}$ were of great relevance, while in relation to $\delta^{13}\text{C}$, which showed a moderate repeatability, differences between nests were of similar order to differences within each nest.

Mean $\delta^{13}\text{C}$ ratios per nest correlated negatively with the proportion of oak trees around the nest-boxes (Table 2, Fig. 2). Prey proportions were unrelated to mean $\delta^{13}\text{C}$ ratios per nest

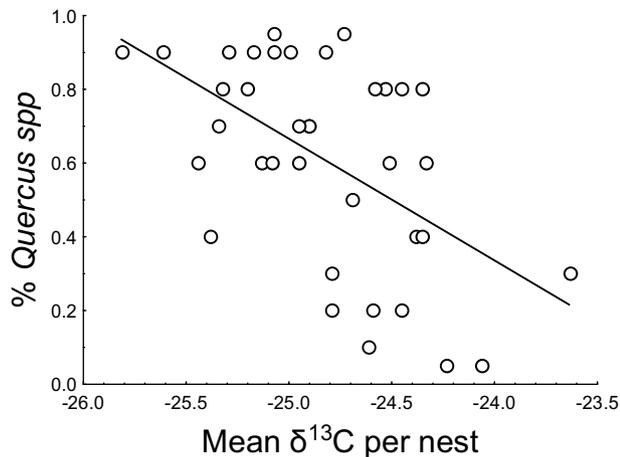


Fig. 2. Relationship between $\delta^{13}\text{C}$ of great tit *Parus major* nestling feathers (mean values per nest) and the proportion of oak trees around the nest-boxes ($r = -0.54$; $P < 0.01$; $N = 37$ nests).

(Table 2). However, mean $\delta^{15}\text{N}$ ratios per nest correlated positively with both the proportion of caterpillars and spiders (Table 2, Fig. 3A and B) and did not correlate with the proportion of oak trees around the nest-boxes (Table 2).

Estimates of prey composition based on Bayesian mixing models correlated strongly with observed prey composition, yet effect sizes were quite low. The estimated proportion of caterpillars from MixSIAR correlated significantly with the proportion of caterpillars estimated from observations (corrected by size) ($r = 0.38$, $P = 0.02$; Fig. 4A), and the estimated proportion of spiders from MixSIAR correlates significantly with the proportion of spiders estimated from observations (corrected by size) ($r = 0.42$, $P = 0.01$; Fig. 4B).

Discussion

Correlations between stable isotopes and observed diet

Regardless of the great variability in prey composition recorded within our study population, mean $\delta^{15}\text{N}$ values per nest of nestling feathers were significantly correlated with the relative abundance of spiders and caterpillars in nestling diet. Conversely, we found that $\delta^{13}\text{C}$ was strongly influenced by habitat structure. As therefore expected, our findings support the view that $\delta^{15}\text{N}$ ratios are a good predictor of an individual's trophic position in the food web (Kelly 2000; Post 2002) and a useful proxy of prey composition (Inger & Bearhop 2008). Data from isotopic ratios of feathers allowed us to estimate the relative intake by nestlings of the two most significant prey items for this species, namely caterpillars and spiders (Pagani-Núñez et al. 2011).

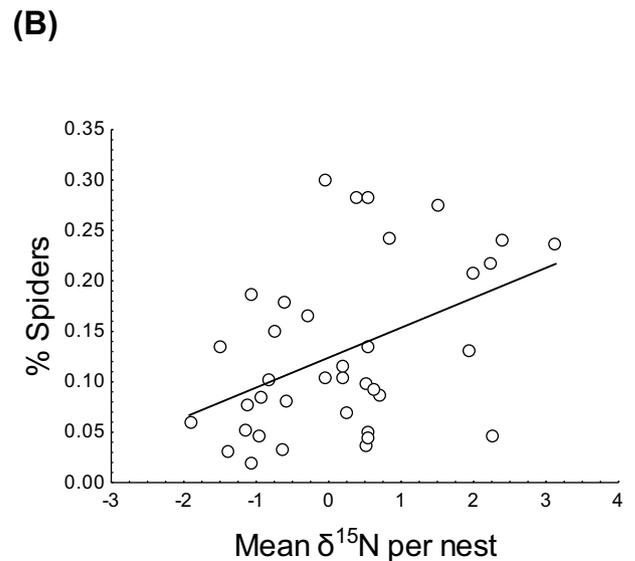
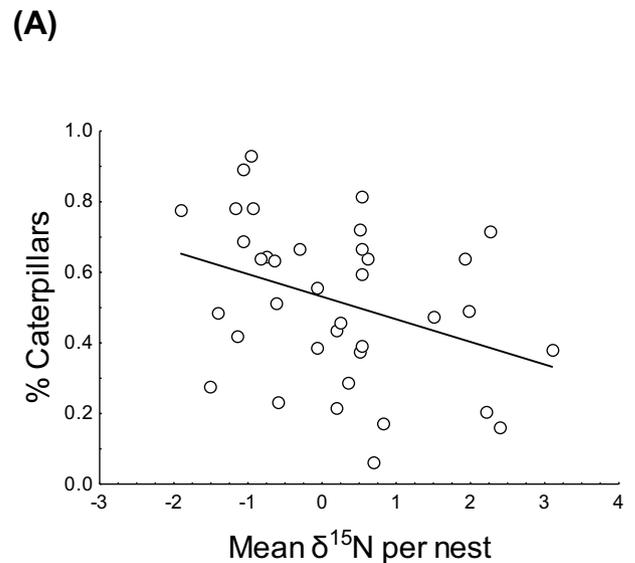


Fig. 3. Relationship between $\delta^{15}\text{N}$ of great tit *Parus major* nestling feathers (mean values per nest) and: (A) the proportion of caterpillars ($r = -0.36$; $P = 0.03$), and (B) the proportions of spiders ($r = 0.44$; $P < 0.01$), in both cases weighted by prey size ($N = 37$ nests).

Correlations between mixing models and observed diet

However, diet estimates based on Bayesian mixing-models did not correlate strongly with the observed diet. There are several potential causes for this mismatch between these two methods. First, in our study population brood size is relatively low and nestling diet is extremely variable among nests, both in relation to habitat structure and the time of breeding (see e.g. Pagani-Núñez & Senar 2014; Pagani-Núñez et al. 2015). Moreover, since diet may vary at different stages of nestling development, this variation may introduce biases at

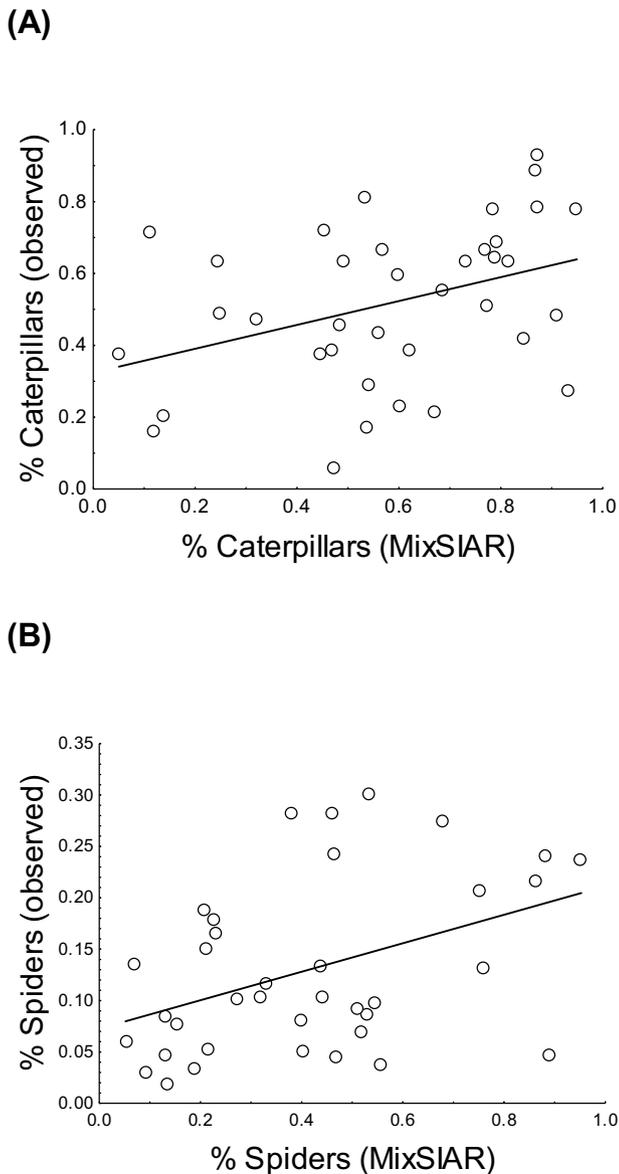


Fig. 4. (A) Relationship between the estimated proportion of caterpillars using MixSIAR and the observed proportion of caterpillars ($r=0.38$; $P=0.02$) and (B) relationship between the estimated proportion of spiders using MixSIAR and the observed proportion of spiders ($r=0.42$; $P=0.01$). Both estimated and observed prey proportions were weighted by prey size ($N=22$ nests).

the moment of estimating prey composition along the whole nesting period based on a single day of observations. Second, many prey types can strongly overlap among them, which could make more difficult to estimate prey proportions. Spiders, which are markedly different from the rest of prey types (McNabb, Halaj, & Wise 2001), can be estimated with more accuracy. Our results stress therefore that different prey types could be estimated with different levels of accuracy (Resano-Mayor et al. 2014).

Two additional factors may be constraining our results. First, we were using prey data from different years to esti-

mate diet proportions of nestling diet. Caterpillars showed a significant difference between years, while spiders did not. Since caterpillars are a very important prey source, this could be another potential confounding factor. Second, our TEFs could be inaccurate, even when using TEFs from closely related species. It has been shown that TEFs are species-specific (McLaren, Crawshaw, & Patterson 2015) and can be affected by diet composition itself (Pearson, Levey, Greenberg, & Martínez del Río 2003; Podlesak & McWilliams 2006). Most enriched diets produce higher carbon and nitrogen TEFs, so in this population with so variable diets these factors could even be different for different broods.

Uncertainties regarding $\delta^{13}\text{C}$

$\delta^{13}\text{C}$ has been widely used as an estimator of trophic niche (Matthews & Mazumder 2004; Araújo et al. 2007; Resano et al. 2011). Accordingly, we found that the best predictor of $\delta^{13}\text{C}$ in nestling feathers was the proportion of *Quercus* spp. surrounding nest boxes. This pattern may be due to a site-effect previously described in other Mediterranean locations (Charmantier et al. 2014). Nevertheless, we should note that differences in $\delta^{13}\text{C}$ within nests were of similar magnitude as differences in $\delta^{13}\text{C}$ between nests, while $\delta^{15}\text{N}$ showed stronger differences between than within nests. Although there is a clear correlation between habitat structure and $\delta^{13}\text{C}$, this high variation within nests suggests that other factors are also contributing to shape this pattern. It is possible that the same prey items had different isotopic signatures according to habitat structure (Girard, Baril, Mineau, & Fahrig 2011, 2012), given the broad ranges of $\delta^{13}\text{C}$ displayed by the different kinds of prey. Hence, if parents preferably feed different individual nestlings with different prey types (even though these preys belong to the same trophic level) this could be a potential source of variation. Interestingly, and in contrast with this study, Charmantier et al. (2014) reported a site-dependent rather than a habitat-dependent pattern for stable isotopes of blue tit feathers. Therefore, we found the paradoxical result that the exploitation of the same prey items in different habitats would account for this pattern of $\delta^{13}\text{C}$ isotopic signatures in nestling feathers, although $\delta^{13}\text{C}$ was not strictly related to main prey proportions. In relation to habitat structure itself, it is possible that these differences in habitat structure affected mean temperatures. In higher and more open areas, where pines predominate, temperatures may be generally higher, while in low and dense areas we found the opposite pattern. This may result in different metabolic rates, which has been shown to influence isotopic incorporation into tissues, especially for $\delta^{13}\text{C}$ (Carleton & del Río 2005).

Conclusions

Further research should identify the main sources of environmental variability of $\delta^{13}\text{C}$ signatures in our area. Our

analysis of repeatability confirmed that habitat-related effects had a relative relevance for isotopic ratios, as within-nest variation in $\delta^{13}\text{C}$ was similar to that between nests. In any case, our results suggest that caution should be exercised when using $\delta^{13}\text{C}$ alone to estimate diet (see e.g. Matthews & Mazumder 2004). This is especially important where environmental heterogeneity is high (Blanco-Fontao, Obeso, Bañuelos, & Quevedo 2012; Girard et al. 2012). Uncertainty can be high if we combine a heterogeneous structure of the habitat with a great diversity of prey. Taking these results into consideration, in order to accelerate and simplify field procedures and in absence of previous information, it could be possible to estimate the degree of herbivory vs. carnivory, or the relative abundance of prey from different trophic levels, based only on stable isotopes data. It could also be possible to determine the habitat in which nestlings were raised, or where they moult, at least on a local scale (Charmantier et al. 2014). Future research will narrow down the environmental sources of variability of isotopic ratios, allowing precise studies on nestling diet using stable isotopes analysis.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.baae.2016.11.004>.

References

- Araújo, M. S., Bolnick, D. I., Machado, G., Giaretta, A. A., & dos Reis, S. F. (2007). Using d^{13}C stable isotopes to quantify individual-level diet variation. *Oecologia*, *152*, 643–654. <http://dx.doi.org/10.1007/s00442-007-0687-1>
- Arnold, K. E., Ramsay, S. L., Donaldson, C., & Adam, A. (2007). Parental prey selection affects risk-taking behaviour and spatial learning in avian offspring. *Proceedings of the Royal Society of London B*, *274*, 2563–2569. <http://dx.doi.org/10.1098/rspb.2007.0687>
- Barba, E., López, J. A., & Gil-Delgado, J. A. (1996). Prey preparation by adult Great Tits *Parus major* feeding nestlings. *Ibis*, *138*, 532–538. <http://dx.doi.org/10.1111/j.1474-919X.1996.tb08074.x>
- Beaulieu, M., & Sockman, K. W. (2012). One meadow for two sparrows: Resource partitioning in a high elevation habitat. *Oecologia*, *170*, 529–540. <http://dx.doi.org/10.1007/s00442-012-2327-7>
- Blanco-Fontao, B., Obeso, J. R., Bañuelos, M. J., & Quevedo, M. (2012). Habitat partitioning and molting site fidelity in *Tetrao urogallus cantabricus* revealed through stable isotopes analysis. *Journal of Ornithology*, *153*, 555–562. <http://dx.doi.org/10.1007/s10336-011-0776-0>
- Boecklen, W. J., Yarnes, C. T., Cook, B. A., & James, A. C. (2011). On the use of stable isotopes in trophic ecology. *Annual Review of Ecology, Evolution, and Systematics*, *42*, 411–440. <http://dx.doi.org/10.1146/annurev-ecolsys-102209-144726>
- Carleton, S. A., & del Rio, C. M. (2005). The effect of cold-induced increased metabolic rate on the rate of ^{13}C and ^{15}N incorporation in house sparrows (*Passer domesticus*). *Oecologia*, *144*, 226–232. <http://dx.doi.org/10.1007/s00442-005-0066-8>
- Charmantier, A., Blondel, J., Perret, P., & Harmelin-Vivien, M. (2014). Tracing site-specific isotopic signatures along a Blue Tit *Cyanistes caeruleus* food chain. *Ibis*, *156*, 165–175. <http://dx.doi.org/10.1111/ibi.12094>
- Cherel, Y., Jaquemet, S., Maglio, A., & Jaeger, A. (2014). Differences in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values between feathers and blood of seabird chicks: Implications for non-invasive isotopic investigations. *Marine Biology*, *161*, 229–237. <http://dx.doi.org/10.1007/s00227-013-2314-5>
- Cross, A. D. P., Hentati-Sundberg, J., Österblom, H., McGill, R. A. R., & Furness, R. W. (2014). Isotopic analysis of island House Martins *Delichon urbica* indicates marine provenance of nutrients. *Ibis*, *156*, 676–681. <http://dx.doi.org/10.1111/ibi.12150>
- Currie, D., Nour, N., & Adriaensen, F. (1996). A new technique for filming prey delivered to nestlings, making minimal alterations to the nest box. *Bird Study*, *43*, 380–382. <http://dx.doi.org/10.1080/00063659609461032>
- David, M., Cézilly, F., & Giraldeau, L. A. (2011). Personality affects zebra finch feeding success in a producer-scrounger game. *Animal Behaviour*, *82*, 61–67. <http://dx.doi.org/10.1016/j.anbehav.2011.03.025>
- Eeva, T., Sillanpää, S., & Salminen, J. P. (2009). The effects of diet quality and quantity on plumage colour and growth of great tit *Parus major* nestlings: A food manipulation experiment along a pollution gradient. *Journal of Avian Biology*, *40*, 491–499. <http://dx.doi.org/10.1111/j.1600-048X.2008.04535.x>
- García-Navas, V., Ferrer, E. S., & Sanz, J. J. (2012). Plumage yellowness predicts foraging ability in the blue tit *Cyanistes caeruleus*. *Biological Journal of the Linnean Society*, *106*, 418–429. <http://dx.doi.org/10.1111/j.1095-8312.2012.01865.x>
- Girard, J., Baril, A., Mineau, P., & Fahrig, L. (2011). Carbon and nitrogen stable isotope ratios differ among invertebrates from field crops, forage crops, and non-cropped land uses. *Ecoscience*, *18*, 98–109. <http://dx.doi.org/10.2980/18-2-3390>

- Girard, J., Baril, A., Mineau, P., & Fahrig, L. (2012). Foraging habitat and diet of Song Sparrows (*Melospiza melodia*) nesting in farmland: A stable isotope approach. *Canadian Journal of Zoology*, *90*, 1339–1350. <http://dx.doi.org/10.1139/z2012-103>
- Gosler, A. G. (1993). *The great tit*. London: Hamlyn.
- Hobson, K. A. (1999). Stable-carbon and nitrogen isotope ratios of songbird feathers grown in two terrestrial biomes: Implications for evaluating trophic relationships and breeding origins. *Condor*, *101*, 799–805. <http://www.jstor.org/stable/1370067>
- Hobson, K. A., & Bairlein, F. (2003). Isotopic fractionation and turnover in captive Garden Warblers (*Sylvia borin*): Implications for delineating dietary and migratory associations in wild passerines. *Canadian Journal of Zoology*, *81*, 1630–1635. <http://dx.doi.org/10.1139/z03-140>
- Inger, R., & Bearhop, S. (2008). Applications of stable isotope analyses to avian ecology. *Ibis*, *150*, 447–461. <http://dx.doi.org/10.1111/j.1474-919X.2008.00839.x>
- Kelly, J. F. (2000). Stable isotopes of carbon and nitrogen in the study of avian and mammalian trophic ecology. *Canadian Journal of Zoology*, *78*, 1–27. <http://dx.doi.org/10.1139/z99-165>
- Krebs, J. R. (1971). Territory and breeding density in the Great Tit *Parus major* L. *Ecology*, *52*, 3–22. <http://www.jstor.org/stable/1934734>
- Margalida, A., Bertran, J., & Boudet, J. (2005). Assessing the diet of nestling Bearded Vultures: A comparison between direct observation methods. *Journal of Field Ornithology*, *76*, 40–45. <http://dx.doi.org/10.1648/0273-8570-76.1.40>
- Matthews, B., & Mazumder, A. (2004). A critical evaluation of intrapopulation variation of $\delta^{13}\text{C}$ and isotopic evidence of individual specialization. *Oecologia*, *140*, 361–371. <http://dx.doi.org/10.1007/s00442-004-1579-2>
- McLaren, A. A. D., Crawshaw, G. J., & Patterson, B. R. (2015). Carbon and nitrogen discrimination factors of wolves and accuracy of diet inferences using stable isotope analysis. *Wildlife Society Bulletin*, *39*, 788–796. <http://dx.doi.org/10.1002/wsb.599>
- McNabb, D. M., Halaj, J., & Wise, D. H. (2001). Inferring trophic positions of generalist predators and their linkage to the detrital food web in agroecosystems: A stable isotope analysis. *Pedobiology*, *45*, 289–297. <http://dx.doi.org/10.1078/0031-4056-00087>
- Michener, R. H., & Lajtha, K. (2007). *Stable isotopes in ecology and environmental science*. Malden: Blackwell Publishing.
- Mizutani, H., Fukuda, M., & Kabaya, Y. (1992). ^{13}C and ^{15}N enrichment factors of feathers of 11 species of adult birds. *Ecology*, *73*, 1391–1395. <http://www.jstor.org/stable/1940684>
- Moreby, S. J., & Stoate, C. H. R. I. (2000). A quantitative comparison of neck-collar and faecal analysis to determine passerine nestling diet. *Bird Study*, *47*, 320–331. <http://dx.doi.org/10.1080/00063650009461192>
- Moreno, R., Jover, L., Munilla, I., Velando, A., & Sanpera, C. (2010). A three-isotope approach to disentangling the diet of a generalist consumer: the yellow-legged gull in northwest Spain. *Marine Biology*, *157*, 545–553. <http://dx.doi.org/10.1007/s00227-009-1340-9>
- Naef-Daenzer, B., & Keller, L. F. (1999). The foraging performance of great and blue tits (*Parus major* and *P. caeruleus*) in relation to caterpillar development, and its consequences for nestling growth and fledging weight. *Journal of Animal Ecology*, *68*, 708–718. <http://dx.doi.org/10.1046/j.1365-2656.1999.00318.x>
- Naef-Daenzer, L., Naef-Daenzer, B., & Nager, R. G. (2000). Prey selection and foraging performance of breeding Great Tits *Parus major* in relation to food availability. *Journal of Avian Biology*, *31*, 206–214. <http://dx.doi.org/10.1034/j.1600-048X.2000.310212.x>
- Nakagawa, S., & Schielzeth, H. (2010). Repeatability for Gaussian and non-Gaussian data: A practical guide for biologists. *Biological Reviews*, *85*, 935–956. <http://dx.doi.org/10.1111/j.1469-185X.2010.00141.x>
- Pagani-Núñez, E., Ruiz, I., Quesada, J., Negro, J. J., & Senar, J. C. (2011). The diet of Great Tit *Parus major* nestlings in a Mediterranean Iberian forest: The important role of spiders. *Animal Biodiversity and Conservation*, *34*, 355–361. <http://www.raco.cat/index.php/ABC/article/view/248927>
- Pagani-Núñez, E., & Senar, J. C. (2013). One hour of sampling is enough: Great tit *Parus major* parents feed their nestlings consistently across time. *Acta Ornithologica*, *48*, 194–200. <http://dx.doi.org/10.3161/000164513X678847>
- Pagani-Núñez, E., & Senar, J. C. (2014). Are colorful males of great tits *Parus major* better parents? Parental investment is a matter of quality. *Acta Oecologica*, *55*, 23–28. <http://dx.doi.org/10.1016/j.actao.2013.11.001>
- Pagani-Núñez, E., Uribe, F., Hernández-Gómez, S., Muñoz, G., & Senar, J. C. (2014). Habitat structure and prey composition generate contrasting effects on carotenoid-based coloration of great tit *Parus major* nestlings. *Biological Journal of the Linnean Society*, *113*, 547–555. <http://dx.doi.org/10.1111/bij.12352>
- Pagani-Núñez, E., Valls, M., & Senar, J. C. (2015). Diet specialization in a generalist population: The case of breeding great tits *Parus major* in the Mediterranean area. *Oecologia*, *179*, 629–640. <http://dx.doi.org/10.1007/s00442-015-3334-2>
- Parnell, A. C., Inger, R., Bearhop, S., & Jackson, A. L. (2010). Source partitioning using stable isotopes: Coping with too much variation. *PLoS One*, *5*, e9672. <http://dx.doi.org/10.1371/journal.pone.0009672>
- Pearson, S., Levey, D., Greenberg, C., & Martínez del Rio, C. (2003). Effects of elemental composition on the incorporation of dietary nitrogen and carbon isotopic signatures in an omnivorous songbird. *Oecologia*, *135*, 516–523. <http://dx.doi.org/10.1007/s00442-003-1221-8>
- Podlesak, D. W., & McWilliams, S. R. (2006). Metabolic routing of dietary nutrients in birds: Effects of diet quality and macronutrient composition revealed using stable isotopes. *Physiological and Biochemical Zoology*, *79*, 534–549. <http://www.jstor.org/stable/10.1086/502813>
- Post, D. M. (2002). Using stable isotopes to estimate trophic position: Models, methods, and assumptions. *Ecology*, *83*, 703–718. [http://dx.doi.org/10.1890/0012-9658\(2002\)083%5B0703:USITET%5D2.0.CO;2](http://dx.doi.org/10.1890/0012-9658(2002)083%5B0703:USITET%5D2.0.CO;2)
- Poulsen, J. G., & Aebischer, N. J. (1995). Quantitative comparison of two methods of assessing diet of nestling Skylarks (*Alauda arvensis*). *Auk*, *112*, 1070–1073. <http://www.jstor.org/stable/4089046>
- Quesada, J., & Senar, J. C. (2007). The role of melanin- and carotenoid-based plumage coloration in nest defence in the Great Tit. *Ethology*, *113*, 640–647. <http://dx.doi.org/10.1111/j.1439-0310.2007.01364.x>
- R Core Team. (2014). *R: A language and environment for statistical computing*. Vienna: R Foundation for Statistical Computing. <http://www.R-project.org/>
- Ramos, R., Ramírez, F., Sanpera, C., Jover, L., & Ruiz, X. (2009). Feeding ecology of yellow-legged gulls *Larus michahellis* in the western Mediterranean: A comparative assessment using

- conventional and isotopic methods. *Marine Ecology Progress Series*, 377, 289–297. <http://dx.doi.org/10.3354/meps07792>
- Redpath, S. M., Clarke, R., Madders, M., & Thirgood, S. J. (2001). Assessing raptor diet: Comparing pellets, prey remains, and observational data at Hen Harrier nests. *Condor*, 103, 184–188. [http://dx.doi.org/10.1650/0010-5422\(2001\)103\[0184:ARDCPP\]2.0.CO;2](http://dx.doi.org/10.1650/0010-5422(2001)103[0184:ARDCPP]2.0.CO;2)
- Resano, J., Hernández-Matías, A., Real, J., & Parés, F. (2011). Using stable isotopes to determine dietary patterns in Bonelli's eagle (*Aquila fasciata*) nestlings. *Journal of Raptor Research*, 45, 342–352. <http://dx.doi.org/10.3356/JRR-11-13.1>
- Resano-Mayor, J., Hernández-Matías, A., Real, J., Parés, F., Inger, R., & Bearhop, S. (2014). Comparing pellet and stable isotope analyses of nestling Bonelli's Eagle *Aquila fasciata* diet. *Ibis*, 156, 176–188. <http://dx.doi.org/10.1111/ibi.12095>
- Robb, G. N., McDonald, R. A., Inger, R., Reynolds, S. J., Newton, J., McGill, R. A., Chamberlain, D. E., Harrison, T. J., & Bearhop, S. (2011). Using stable-isotope analysis as a technique for determining consumption of supplementary foods by individual birds. *Condor*, 113, 475–482. <http://dx.doi.org/10.1525/cond.2011.090111>
- Royama, T. (1966). Factors governing feeding rate, food requirement and brood size in nestling great tits (*Parus major*). *Ibis*, 108, 313–347. <http://dx.doi.org/10.1111/j.1474-919X.1966.tb07348.x>
- Rytkonen, S., & Krams, I. (2003). Does foraging behaviour explain the poor breeding success of great tits *Parus major* in northern Europe? *Journal of Avian Biology*, 34, 288–297. <http://dx.doi.org/10.1034/j.1600-048X.2003.03041.x>
- StatSoft. (2001). *STATISTICA data analysis software system, version 6.0*. Tulsa: StatSoft Inc.
- Stephens, D. W., Brown, J. S., & Ydenberg, R. C. (2007). *Foraging: Behavior and ecology*. Chicago: The University of Chicago Press.
- Stock, B. C., & Semmens, B. X. (2013). *MixSIAR GUI User Manual version 1.0*. <http://conserver.iugo-cafe.org/user/brice.semmens/MixSIAR>.
- Sweeting, C. J., Polunin, N. V. C., & Jennings, S. (2006). Effects of chemical lipid extraction and arithmetic lipid correction on stable isotope ratios of fish tissues. *Rapid Communications in Mass Spectrometry*, 20, 595–601. <http://dx.doi.org/10.1002/rcm.2347>
- Tremblay, I., Thomas, D., Blondel, J., Perret, P., & Lambrechts, M. M. (2005). The effect of habitat quality on foraging patterns, provisioning rate and nestling growth in Corsican Blue Tits *Parus caeruleus*. *Ibis*, 147, 17–24. <http://dx.doi.org/10.1111/j.1474-919x.2004.00312.x>
- Vitz, A. C., & Rodewald, A. D. (2012). Using stable isotopes to investigate the dietary trophic level of fledgling songbirds. *Journal of Field Ornithology*, 83, 73–84. <http://dx.doi.org/10.1111/j.1557-9263.2011.00357.x>
- Votier, S. C., Bearhop, S., MacCormick, A., Ratcliffe, N., & Furness, R. W. (2003). Assessing the diet of great skuas, *Catharacta skua*, using five different techniques. *Polar Biology*, 26, 20–26. <http://dx.doi.org/10.1007/s00300-002-0446-z>

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