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]

[a]Evolutionary and Behavioural Ecology Research Unit, Natural History Museum of Barcelona, Psg. Picasso s/n., 08003 Barcelona, Spain
[b]Behavioral and Community Ecology, Conservation Biology Group, College of Forestry, Guangxi University, No. 100 Daxue Road, Nanning, Guangxi 530005, People's Republic of China
[c]Department of Integrative Biology, University of Texas, Austin, USA
[d]Department of Collective Behaviour, Max Planck Institute for Ornithology, Germany
[e]Department of Animal Biology, University of Barcelona, Av. Diagonal 643, 08028 Barcelona, Spain

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 $\delta^{13}C$ and $\delta^{15}N$ 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 $\delta^{15}N$ were larger than within-nest differences. We found that $\delta^{15}N$ 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 $\delta^{13}C$ ratios were of similar magnitude and $\delta^{13}C$ 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).

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

Die Unterschiede bei den δ^{15}N-Signaturen waren zwischen den Nestern größer als innerhalb der Nester. Die δ^{15}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 δ^{13}C-Verhältnisse zwischen und innerhalb der Nester von gleicher Größenordnung, und die δ^{13}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 Nahrungsmusteer festgestellt werden (z.B. durch störende Einflüsse von Habitat und/oder zeitlicher Variation), kann es schwierig werden, sichere Schlüsse hinsichtlich der Nahrungszusammensetzung zu ziehen.

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, δ^{13}C (^{13}C/^{12}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). δ^{15}N (^{15}N/^{14}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,