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Comparing pellet and stable isotope analyses of nestling Bonelli's Eagle *Aquila fasciata* diet

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Diet analyses are central to the study of avian trophic ecology, and stable isotope analyses have made an increasing contribution in the last two decades. Few isotopic studies have assessed the diet of raptor species, which are more frequently analysed by conventional diet methods such as pellet analysis. In this study, we compare prey consumption estimates of nestling Bonelli's Eagles *Aquila fasciata* from conventional pellet analysis (in terms of items and biomass) and stable isotopic mixing models (SIAR) using $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$ of feathers. The pellet analysis showed that European Rabbits *Oryctolagus cuniculus*, pigeons (mainly Common Wood Pigeons *Columba palumbus* and Domestic Pigeons *Columba livia dom.*), Red-legged Partridges *Alectoris rufa*, passerines, Yellow-legged Gulls *Larus michahellis* and Eurasian Red Squirrels *Sciurus vulgaris* were the main prey, so they were selected for diet reconstructions in SIAR. At the population level, mean prey consumption estimates were similar for pellets (both items and biomass) and SIAR. At the territory level, the weighted kappa statistic showed good ordinal scale agreement in main prey consumption between items or biomass and SIAR. Although the intraclass correlation coefficient showed poor method agreement when considering all prey in the same analysis, the intraclass correlation coefficients for each prey category showed significant agreement between pellets and SIAR when estimating the consumption of Rabbits, pigeons and Gulls, with lower agreement for passerines and Squirrels. Lastly, there was poor method agreement for estimates of Partridges. Our results suggest an overall agreement between the pellet analysis and SIAR when estimating nestling Bonelli's Eagle diet at both the population and, to a lesser extent, the territory level, supporting the usefulness of isotopic mixing models when identifying the terrestrial and marine components of raptor diets.

Keywords: carbon isotopes, conventional diet analysis, foraging ecology, isotopic mixing models, nitrogen isotopes, predators, raptors, sulphur isotopes.

Animal foraging ecology explains much of the observed variation among individual fitness correlates such as body condition, survival and breeding success (Schoener 1971, Pyke 1984, Inger *et al.* 2008, Terraube *et al.* 2012). As such, it can also explain population dynamics, prey–predator relationships and species distributions (Newton 1998, Moleón *et al.* 2009, Cortés-Avizanda *et al.* 2011). Nevertheless, measuring diet is often challenging due to the difficulty of making direct observations of feeding events over long periods, with consequent reliance on indirect methods and their potential biases (Real 1996, Votier *et al.* 2003, Huang *et al.* 2006).

The most common methods of diet assessment in birds are direct observations of feeding habits and analyses of nest food remains, individual

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stomach contents, faecal droppings and regurgitated pellets (Marti *et al.* 2007, Maziarz & Wesolowski 2010, Michalski *et al.* 2011, Bourass *et al.* 2012), although these methods have a number of limitations (Real 1996, Votier *et al.* 2003). For instance, they usually involve a great effort in terms of data collection and analysis. Moreover, they often reflect only a snapshot of a consumer's diet (Inger & Bearhop 2008) and present potential biases linked to prey sizes or digestibility (Brown & Ewins 1996, Real 1996, Votier *et al.* 2003, Marti *et al.* 2007).

Over the last two decades, the use of stable isotope analysis (SIA) to study avian trophic ecology has increased considerably (Kelly 2000, Inger & Bearhop 2008, Hobson 2011). The isotopic ratios in bird tissue reflect its diet at the time of tissue synthesis in a predictable manner. The shift in isotope ratio between diet and consumer tissue is known as the trophic enrichment factor (TEF) and can be used in isotopic mixing models to quantify the relative contributions of isotopically distinct sources to the diet of individuals or populations (Inger *et al.* 2006, Moreno *et al.* 2010). More recently, Bayesian isotopic mixing models have been developed to account for uncertainty and variation in model estimates, allow for multiple dietary sources, and generate potential dietary solutions as true probability distributions (Moore & Semmens 2008, Jackson *et al.* 2009, Parnell *et al.* 2010). Nevertheless, the use of isotopic mixing models requires accurate prior information regarding the trophic ecology of the studied species, and dietary estimates from mixing models would be only as good as the assumptions and parameters on which they depend (Bond & Diamond 2011, Hobson 2011).

Despite the applicability of both conventional diet analyses and isotopic mixing models to determine avian diets, and the caveats and potential biases associated with each, few studies have compared these methods (but see Doucette *et al.* 2011, Steenweg *et al.* 2011, Weiser & Powell 2011). Moreover, although conventional methods have been used traditionally to assess raptor food habits (Real 1996, Marti *et al.* 2007, Sánchez *et al.* 2008, Bakaloudis *et al.* 2012), to date few isotopic studies have focused on assessing the diets of avian terrestrial predators, including most raptor species (but see Roemer *et al.* 2002, Caut *et al.* 2006). Consequently, the potential applicability of isotopic mixing models to assess raptor foraging ecology is still poorly understood. The fact that isotopic data inform about assimilated rather than just ingested prey is a major advantage of using isotopic analysis to study raptor diet. Moreover, isotopic mixing models provide a powerful tool to estimate the foraging ecology of individuals to test the incidence and implications of individual resource use (Bolnick *et al.* 2003). Finally, isotopic analysis may constitute a homogeneous sampling procedure to monitor temporal or spatial variation in raptor diets.

Bonelli's Eagle *Aquila fasciata* is distributed from the western Mediterranean to southeast Asia (del Hoyo *et al.* 1994). The European population is now classified as endangered after a marked decline in number and range in recent decades (BirdLife International 2004), related to unnaturally high mortality rates, habitat degradation and decline of their main prey species (Real 2004, Hernández-Matías *et al.* 2011). The diet of Bonelli's Eagle in the Mediterranean has been widely studied by conventional methods, showing that the species mainly predates European Rabbits *Oryctolagus cuniculus*, partridges *Alectoris* spp., pigeons *Columba* spp., passerines (mainly corvids and thrushes) and lizards (Real 1991, Moleón *et al.* 2009). More recently, an isotopic approach showed that $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$ are useful to assess both terrestrial and marine prey consumption of Bonelli's Eagle nestlings (Resano *et al.* 2011). Consequently, this species is a suitable model to test whether conventional diet analysis and isotopic mixing models provide similar information when assessing the diet of avian predators.

The aim of this study was to compare prey consumption estimates of nestling Bonelli's Eagles using conventional pellet analysis and Bayesian isotopic mixing models by (1) performing a comprehensive pellet analysis in terms of prey item consumption and prey assimilated biomass, (2) characterizing the isotopic composition of main prey types and (3) comparing main prey consumption estimates obtained from the pellet analysis and the isotopic mixing models.

METHODS

Study area and data collection

From 2008 to 2010 we monitored 43 successful breeding attempts of 28 territorial pairs of Bonelli's Eagle in Catalonia (41°20′N, 01°32′E). Habitat characteristics differed between territories but all

showed Mediterranean landscape features (Carrascal & Seoane 2009, Bosch *et al.* 2010), with an average annual rainfall ranging from 425 to 664 mm. All sampled nests were located on cliffs, and the altitude of nesting areas ranged from 30 to 776 m asl.

From January to early March, we monitored breeding territories to assess occupancy and breeding activity. In late March and April, occupied nests were checked to detect the number of nestlings and their age, which was estimated by feather development and laying date (Real 1991, Gil-Sánchez 2000). To minimize the risk of disturbance, observations were always carried out from long distances using 10× binoculars and 20–60× spotting scopes. Once nestlings were approximately 37 days old, we caught them with the assistance of experienced climbers and sampled three to four mantle feathers from each individual for SIA. Pellets were collected from the nests after the breeding season and analysed to determine nestling diet by conventional methods (Real 1996).

To characterize isotopically the main prey of Bonelli's Eagle, we collected muscle samples from 215 individuals of the following species or species groups during 2008–2011: European Rabbits (n = 42), Red-legged Partridges *Alectoris rufa* (n = 38), Common Wood Pigeons *Columba palumbus* (n = 39), Domestic Pigeons *Columba livia dom.* (n = 45), passerines (Corvidae, Sturnidae and Turdidae; n = 40), Yellow-legged Gulls *Larus michahellis* (n = 4) and Eurasian Red Squirrels *Sciurus vulgaris* (n = 7). All individuals were obtained from the studied Eagles' breeding territories, either in the nests or their surroundings, except most passerines and some Squirrels, which came from rehabilitation centres located in the study area.

Pellet analysis

Each prey item identified in each pellet was counted as one item (Real 1996, Gil-Sánchez *et al.* 2004). Pellet contents (i.e. feathers, bones, hair, nails and scales) were identified with the help of a reference collection, a 4× magnifying glass and consulting specialized guides (Brom 1986, Brown *et al.* 2003). Prey items were identified to species level whenever possible.

Prey consumption was estimated for any given territory and year (hereafter referred to as territory level; n = 43) as percentages of total items and total biomass, as is common in raptor diet studies (Real 1996, Sánchez-Zapata & Calvo 1998). To calculate the biomass of each prey type we used the weights of each prey species, corrected for the degree of consumption by adults at the nest before delivering prey to the chicks. Mean weights of prey species were obtained from the literature (Brom 1986, Real 1991, del Hoyo *et al.* 1997), most estimates being from measurements of individuals from the study area (see Supporting Information Table S1). Consumption of each prey type was estimated on the basis of field observations of feeding events from a hide (n = 182 prey items; J. Real unpubl. data). Therefore our final prey net biomass estimates (Table S1) were representative of the nestlings' ingested biomass rather than total prey biomass. More than 20 prey items in each territory and year were included to ensure reliability in the pellet analysis (Ontiveros *et al.* 2005).

Based on the results of pellet analysis, the most-consumed prey categories in terms of items or biomass (hereafter main prey categories) were selected for comparison using the two diet assessment methods: European Rabbits, pigeons (mostly Common Wood Pigeons and Domestic Pigeons), Red-legged Partridges, passerines, Yellow-legged Gulls and Eurasian Red Squirrels. Main prey consumption values from either the items or the biomass approaches were re-scaled relative to their global percentage in each territory to ensure that main prey categories accounted for the 100% of the diet in each territory. These re-scaled values were used for comparison with estimates obtained from the isotopic mixing models.

Prey isotopic characterization

Isotopic signatures of species may be influenced by their local environment (Connolly *et al.* 2004, Choi *et al.* 2007) and hence isotopic values of main prey categories may differ between Bonelli's Eagle territories. To test this, prey samples of the most widely consumed prey (Rabbits, pigeons and Partridges) were characterized as a function of proximity to the sea and to habitat in the Eagle territory from which they came. Territories located on coastal cliffs or in coastal mountain ranges were classified as marine, and those farther inland as terrestrial. Territory habitat was measured in a 3.3-km radius around the nest to represent the home-range used by Bonelli's Eagles (Bosch *et al.*

2010), and each territory was classified according to its predominant habitat as either forest, scrubland or agricultural. Habitat predominance was estimated using MiraMon v6.4 software (Pons 2002) from land cover data available at a scale of 1 : 3000 and updated in December 2009.

Stable isotope analysis

A 3-cm^3 piece of muscle from the chest or leg of each sampled prey animal was lyophilized for 48 h. Samples were lipid-extracted using several chloroform–methanol (2 : 1) rinses following Folch *et al.* (1957). Muscle was ground into fine powder using an impactor mill (Freezer/mill 6750 Spex Certiprep) and subsamples of approximately 0.32 mg (for $\delta^{13}C$ and $\delta^{15}N$) and 5.6 mg (for $\delta^{34}S$) were loaded in tin receptacles and crimped for combustion. Nestling feathers were first cleaned in a solution of NaOH (0.25 M) and oven-dried at 40 °C for 24 h. Feathers were ground into fine powder and subsamples of 0.35 mg (for $\delta^{13}C$ and $\delta^{15}N$) and 3.7 mg (for $\delta^{34}S$) were loaded in tin receptacles before combustion. Isotopic measurements of both prey and nestlings were performed at the Scientific and Technological Centres of the University of Barcelona using the methods of Resano *et al.* (2011).

Stable isotope ratios are reported as δ values and expressed in ‰, according to the following equation: $\delta X = [(R\text{sample}/R\text{standard}) - 1] \times 1000$, where X is ^{13}C, ^{15}N or ^{34}S and R is the corresponding ratio $^{13}C/^{12}C$, $^{15}N/^{14}N$ or $^{34}S/^{32}S$. *R*standard is the ratio of the international standards: Pee Dee Belemnite (PDB) for ^{13}C, atmospheric nitrogen (AIR) for ^{15}N and Canyon Diablo Troilite (CDT) for ^{34}S. Measurement precisions for $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$ were ≤ 0.15‰, ≤ 0.25‰ and ≤ 0.40‰, respectively.

Bayesian isotopic mixing models

We used the SIAR package for R (Parnell *et al.* 2010) to estimate the relative contribution of main prey categories to the diet of Bonelli's Eagle nestlings at the territory level. $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$ from nestlings and prey were included in the models. Each nest and year was considered a single statistical observation by estimating the mean isotopic values of sampled siblings. Prey isotopic values were selected for each territory: either the overall mean prey values when no effect of environmental features on prey signature was detected, or different values for a single prey when their isotopic values were affected by environmental features (see below). The TEFs for $\delta^{13}C$ (2.1‰ ± 0.08 sd) and $\delta^{15}N$ (2.7‰ ± 0.5 sd) were those obtained for feathers of Peregrine Falcons *Falco peregrinus* fed on muscle of Japanese Quail *Coturnix japonica* (Hobson & Clark 1992). We selected those values because the consumer in that experiment was taxonomically related to our consumer species, and the tissues analysed from both consumers and prey also matched those we studied. The TEF for $\delta^{34}S$ (0‰ ± 0.5 sd) was also obtained from the literature (Michener & Lajtha 2007), where it is commonly assumed that there is no enrichment in ^{34}S in animal diets. Common Wood Pigeon and Domestic Pigeon were included as separate sources within the models, and their consumption estimates from SIAR were summed *a posteriori* to allow for direct comparison with the pellet data.

Statistical analyses

Prey isotopic data were checked for departures from normality using the Kolmogorov–Smirnov test and Q–Q plots. We performed a multivariate analysis of variance (MANOVA) to assess whether prey isotopic values ($\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$ as the dependent variables) differed by species (fitted as a single factor with Rabbits, Common Wood Pigeons, Domestic Pigeons, Partridges, passerines, Gulls and Squirrels as the group categories; $n = 215$). Additionally, we performed a second MANOVA to assess the effect of species, sea proximity and habitat (fitted as fixed effects) on prey isotopic values, but only including the most widely consumed prey (Rabbits, Common Wood Pigeons, Domestic Pigeons and Partridges; $n = 164$). Passerines, Gulls and Squirrels were excluded from this analysis because they were rare in some territory categories. Those factors with a significant effect in the MANOVAs were subjected to one-way analysis of variance (ANOVA) to test the factor effect on each dependent variable ($\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$) separately (Quinn & Keough 2002). Levene's test was used to detect heteroscedasticity and Welch's correction was applied accordingly. Post-hoc pairwise analysis included Tukey's procedure or the Tamhane test when variances were heterogeneous. Prey isotopic values are reported as means ± sd.

To estimate prey consumption at the population level by the pellet analysis, we first calculated mean diet for each territory ($n = 28$), and then a mean was calculated across all territories. Re-scaled values of the main prey categories (for both items and biomass) and SIAR estimates were used for method comparisons ($n = 43$ territory-years) both at the population and the territory levels. These prey consumption percentages were arcsine-transformed and checked for normality using the Kolmogorov–Smirnov test and Q–Q plots. At the population level, a two-way ANOVA was used to assess whether prey consumption estimates differed by prey category and dietary method, with prey consumption estimates from all territories as the dependent variable and both prey category and dietary method as fixed factors. Additionally, separate one-way ANOVAs were used to test the method effect on each prey category. At the territory level, the weighted kappa statistic (Kw) was used to assess agreement between methods on an ordinal scale by ordering main prey categories from higher to lower rates of consumption, and the intraclass correlation coefficient (ICC) was used to test for the agreement of two methods in their quantitative prey consumption estimates. In this regard, we first calculated the ICCs using a three-way mixed effects model (Zhou *et al.* 2011) with prey consumption values as the dependent variable, territory and method as random effects, and prey category as a fixed factor. Bland–Altman plots (Bland & Altman 1986) were created to represent the method's repeatability in prey estimates. Secondly, ICCs were calculated by a two-way mixed effects model (McGraw & Wong 1996) for each prey category.

Statistical analyses were conducted using SPSS 15.0 (SPSS, Chicago, IL, USA) and MEDCALC 12.3.0 (MedCalc Software, Mariakerke, Belgium). SIAR was run using R software (R Development Core Team 2007).

RESULTS

Pellet analysis

We identified 2254 prey items in the 979 pellets analysed, corresponding to at least 31 prey species (Table S1). Birds accounted for 59.3%, mammals for 33.6% and reptiles for 7.1% of prey items, and 55.2%, 40.8% and 4% of biomass, respectively. At the population level, the most frequently consumed prey were pigeons (26.3%), Rabbits (21.1%), passerines (10.7%) and Red-legged Partridges (10.6%), which together accounted for 68.7% of dietary items. In terms of biomass, Rabbits were the main prey item (30.9%), followed by pigeons (26.9%), Yellow-legged Gulls (8.7%), Partridges (8.1%) and Squirrels (4.9%), together accounting for 79.5% of total biomass ingested.

Prey isotopic characterization

There was a significant difference between main prey items in isotopic values (MANOVA: Wilks' lambda, $F_{18,583} = 17.56$, $P < 0.001$). There were overall differences between prey categories in $\delta^{13}C$ (one-way ANOVA: $F_{\text{Welch } 6,30} = 48.63$, $P < 0.001$), but Red-legged Partridges, Common Wood Pigeons and passerines, and Domestic Pigeons, Eurasian Red Squirrels and Yellow-legged Gulls formed two sub-groups of prey within which pairwise differences were not significant. Overall significant differences in $\delta^{15}N$ (one-way ANOVA: $F_{8,206} = 22.20$, $P < 0.001$) were related to prey trophic level. For instance, $\delta^{15}N$ in rabbits was significantly lower than in other prey except Squirrels, and Yellow-legged Gulls had significantly higher $\delta^{15}N$ than most prey. Fewer pairwise differences in $\delta^{15}N$ were found between Squirrels, Partridges, pigeons or passerines. Yellow-legged Gulls showed the highest $\delta^{34}S$ values, and most of the significant differences in $\delta^{34}S$ (one-way ANOVA: $F_{8,36} = 7.45$, $P < 0.001$) seemed to be related to a marine influence (see below). Mean prey isotopic values included in the isotopic mixing models are summarized in Table 1. Isotope biplots ($\delta^{13}C$, $\delta^{15}N$, $\delta^{34}S$) showed that Bonelli's Eagle nestlings lay within the space delineated by main prey categories previously corrected by TEFs (Fig. 1).

In the second MANOVA we found a significant overall effect of species (MANOVA: Wilks' lambda, $F_{9,355} = 16.11$, $P < 0.001$), sea proximity (MANOVA: Wilks' lambda, $F_{3,146} = 9.92$, $P < 0.001$) and habitat (MANOVA: Wilks' lambda, $F_{6,292} = 7.90$, $P < 0.001$) on isotopic prey values, with a significant interaction between species and both sea proximity (MANOVA: Wilks' lambda, $F_{9,355} = 3.97$, $P < 0.001$) and habitat (MANOVA: Wilks' lambda, $F_{18,413} = 1.78$, $P < 0.05$). European Rabbits and Domestic Pigeons from marine territories had higher $\delta^{34}S$ than those from terrestrial territories (one-way ANOVA: $F_{1,40} = 21.12$, $P < 0.001$ and $F_{1,43} = 19.28$, $P < 0.001$, respectively; Fig. 2). Moreover, Domestic Pigeons from agricultural terri-

Table 1. $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$ (mean ± sd; ‰) in main prey types of Bonelli's Eagle in Catalonia: European Rabbits (OC), Common Wood Pigeons (CP), Red-legged Partridges (AR), Domestic Pigeons (CL), passerines (PAS), Yellow-legged Gulls (LM) and Eurasian Red Squirrels (SV). Within prey types, significant values differing either by sea proximity, habitat or both, are shown in bold type. Samples from marine or terrestrial territories are shown as (m) or (t), respectively. Samples from different habitat types are shown as forest (1), scrubland (2) or agricultural (3). Mean prey values were considered when there was no significant influence of environmental features. Prey $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$ values listed in this table were those included in the SIAR, accordingly selected for each territory depending on their sea proximity or habitat type.

Prey	$\delta^{13}C$	$\delta^{15}N$	$\delta^{34}S$
OCm	–25.33 ± 1.02	3.17 ± 2.50	**8.81 ± 2.62**
OCt	–25.33 ± 1.02	3.17 ± 2.50	**5.22 ± 2.07**
CP	–23.98 ± 0.88	5.54 ± 1.75	5.33 ± 1.68
AR	–23.32 ± 1.30	5.81 ± 1.77	6.10 ± 2.59
CLm_1	–21.28 ± 3.88	7.15 ± 2.03	**6.38 ± 0.73**
CLt_1	–21.28 ± 3.88	7.15 ± 2.03	**4.74 ± 1.34**
CLm_2	–21.28 ± 3.88	**6.85 ± 0.77**	**6.38 ± 0.73**
CLt_2	–21.28 ± 3.88	**6.85 ± 0.77**	**4.74 ± 1.34**
CLm_3	–21.28 ± 3.88	**8.06 ± 1.37**	**6.38 ± 0.73**
CLt_3	–21.28 ± 3.88	**8.06 ± 1.37**	**4.74 ± 1.34**
PAS	–23.36 ± 0.72	7.25 ± 1.24	6.51 ± 0.90
LM	–20.50 ± 0.55	9.35 ± 0.55	11.97 ± 3.88
SV	–19.36 ± 1.60	3.23 ± 1.99	5.47 ± 2.08

tories had higher $\delta^{15}N$ than those from scrubland territories (one-way ANOVA: $F_{\text{Welch } 2,27} = 4.60$, $P < 0.05$; Fig. 2). On the other hand, isotopic values of Rabbits were not influenced by habitat, and neither sea proximity nor habitat features influenced isotopic values of Common Wood Pigeons or Red-legged Partridges.

Comparison of pellet analysis and SIAR

At the population level, mean consumption estimates differed by prey category (two-way ANOVA: $F_{5,756} = 145.76$, $P < 0.001$) and dietary method (two-way ANOVA: $F_{2,756} = 8.01$, $P < 0.001$). Although there was a significant interaction between prey category and the dietary method effects (two-way ANOVA: $F_{10,756} = 4.38$, $P < 0.001$), all methods estimated a similar dietary pattern of higher consumption of Rabbits and pigeons, and lower consumption of Partridges, passerines, Gulls and Squirrels (Fig. 3, Supporting Information Table S2). When comparing methods for each prey category (i.e. prey item counts from pellets vs. biomass estimation from pellets vs. SIAR), there were significant differences between

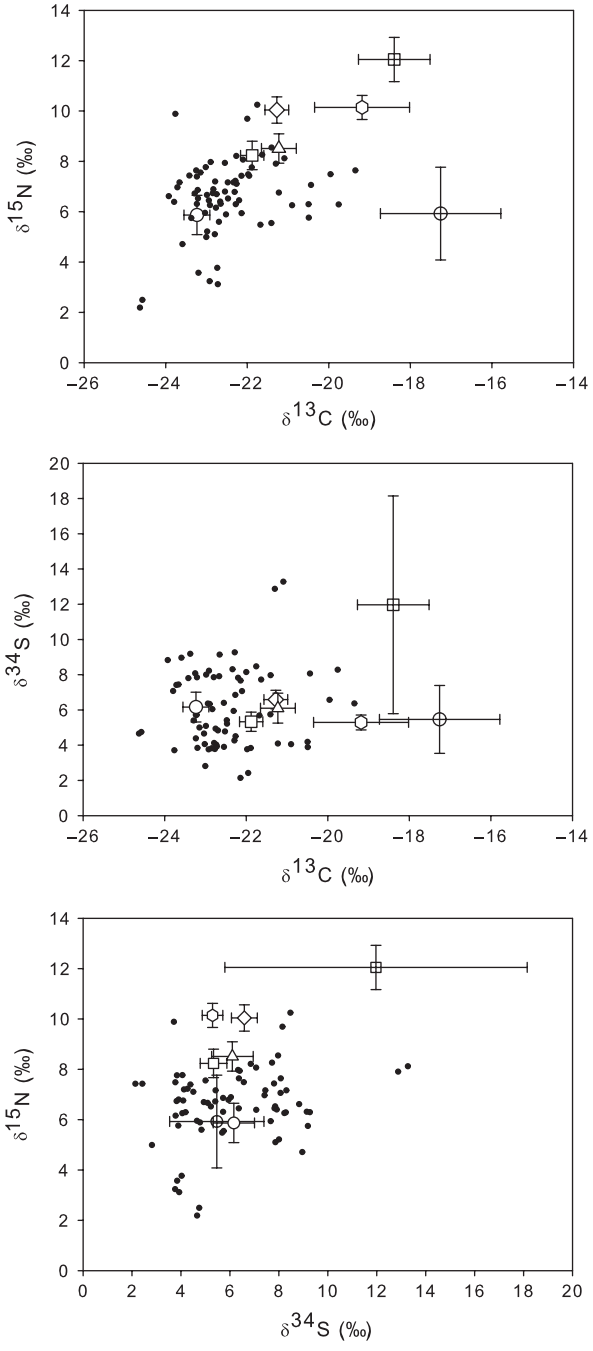

Figure 1. Isotopic values ($\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$) of Bonelli's Eagle nestlings and main prey types (mean ± 95% CI) in Catalonia. Open symbols represent main prey types: European Rabbits (open circle), Common Wood Pigeons (open square), Red-legged Partridges (open triangle), Domestic Pigeons (open hexagon), passerines (open rhombus), Yellow-legged Gulls (crossed square) and Eurasian Red Squirrels (crossed circle). Closed dots represent Bonelli's Eagle nestlings. Prey isotopic values are corrected by TEFs (2.1‰ for $\delta^{13}C$, 2.7‰ for $\delta^{15}N$ and 0‰ for $\delta^{34}S$).

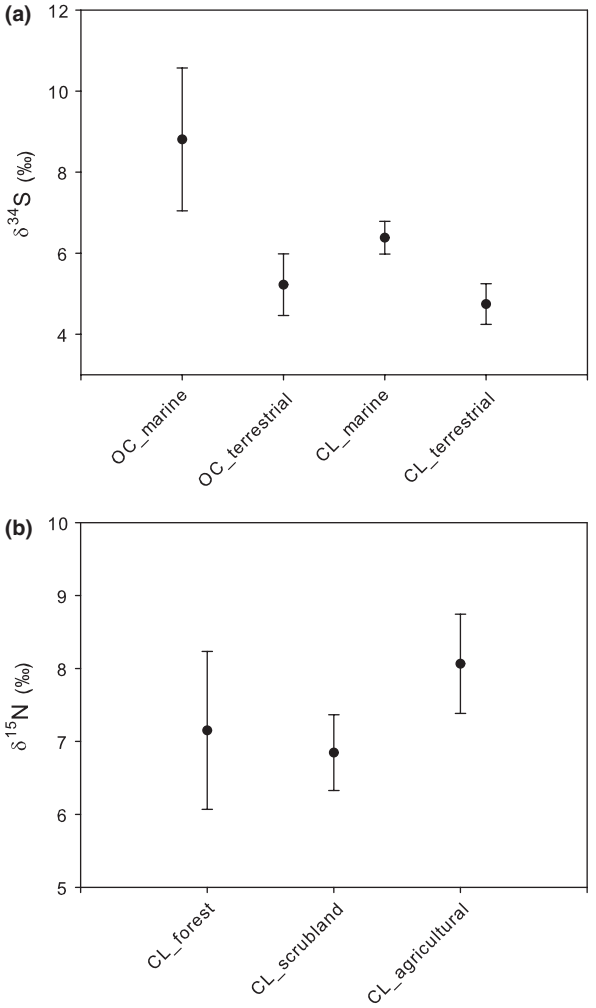

Figure 2. Influence of territorial environmental features on prey isotopic values. (a) Significant differences for $\delta^{34}S$ in European Rabbits (OC) and Domestic Pigeons (CL) from marine and terrestrial territories. (b) Significant differences for $\delta^{15}N$ in Domestic Pigeons (CL) from territories with scrubland and agricultural predominance. Domestic Pigeons from territories with forest predominance are shown, although they were not significantly different from any other habitat category. Prey isotopic values are represented as mean ± 95% CI.

prey item counts and biomass estimation in Rabbits (one-way ANOVA: $F_{2,126} = 4.47$, $P < 0.05$), between biomass estimation and SIAR in Partridges (one-way ANOVA: $F_{\text{Welch } 2,64} = 9.09$, $P < 0.001$), between biomass estimation and both prey item counts and SIAR in passerines (one-way ANOVA: $F_{\text{Welch } 2,64} = 22.06$, $P < 0.001$), between prey item counts and SIAR in Gulls (one-way ANOVA: $F_{\text{Welch } 2,67} = 12.80$, $P < 0.001$), and between biomass estimation and SIAR in Squirrels (one-way ANOVA: $F_{\text{Welch } 2,68} = 3.87$, $P < 0.05$).

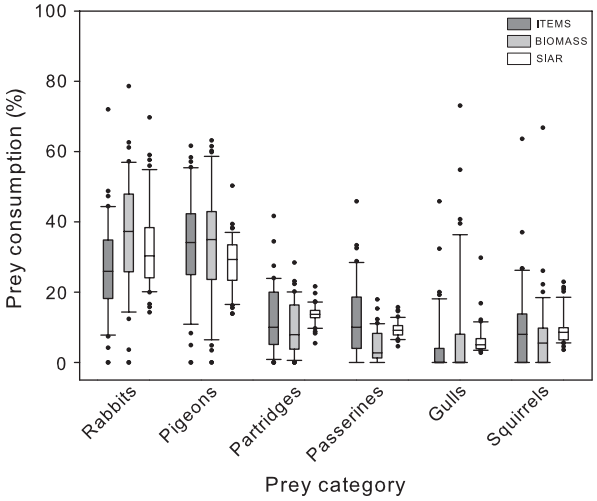

Figure 3. Boxplot with the consumption of main prey categories estimated by ITEMS (dark grey), BIOMASS (light grey) and SIAR (white) at the population level.

At the territory level, we found good agreement when ordering main prey categories from higher to lower levels of consumption between both prey item counts and SIAR (Kw = 0.47, 95% CI = 0.40–0.54) and between biomass estimation and SIAR (Kw = 0.53, 95% CI = 0.46–0.59). In both comparisons, the highest agreement was found when estimating the most- or least-consumed prey categories (1 or 6), with lower agreement for the other prey categories (Fig. 4).

The overall ICC showed low agreement among prey estimates when comparing prey item counts and SIAR (ICC = 0.30, $P = 0.13$) or biomass estimation and SIAR (ICC = 0.29, $P = 0.14$; Table 2). Bland–Altman plots illustrated a significant positive correlation between the difference in prey estimates between methods (items – SIAR or biomass – SIAR) and the mean prey consumption values obtained from both methods ($r_s = 0.35$, $P < 0.001$ for prey item counts vs. SIAR and $r_s = 0.47$, $P < 0.001$ for biomass estimation vs. SIAR; Fig. 5). In other words, the pellet analysis (in terms of both items and biomass) estimated lower consumption rates than SIAR for less-consumed prey, whereas the opposite was true for more-consumed prey.

When assessing agreement between methods for each prey category, we found agreement between prey item counts and SIAR estimates for Rabbits (ICC = 0.42, $P < 0.05$), pigeons (ICC = 0.44, $P < 0.05$) and Gulls (ICC = 0.55, $P < 0.01$), but no significant agreement between methods for passerines (ICC = 0.21, $P = 0.22$) or Squirrels

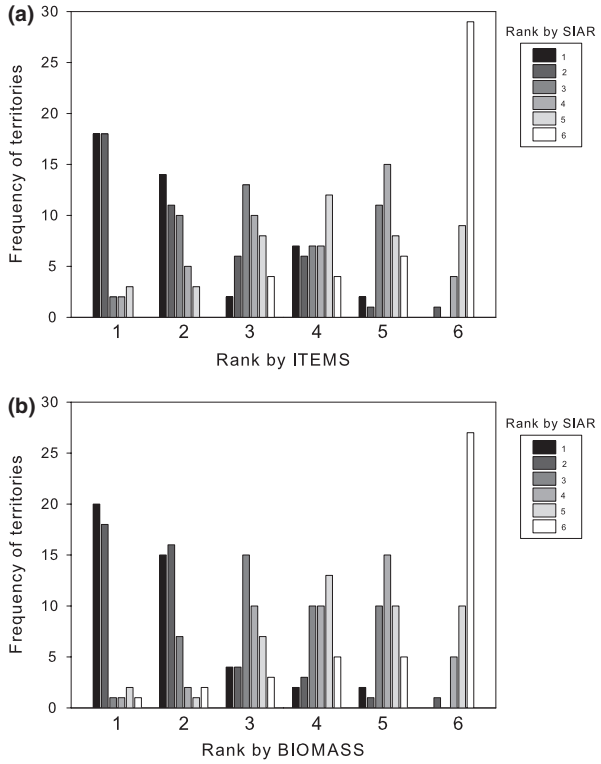

Figure 4. Agreement between ITEMS and SIAR (a) or BIOMASS and SIAR (b) in main prey consumption estimates when these are ranked in an ordinal scale (from higher to lower consumption) at the territory–year level. For the ITEMS and BIOMASS approaches, main prey categories are scaled from higher (1) to lower (6) consumption. According to SIAR, prey categories are scaled from higher (black) to lower (white) consumption. The *x*-axis shows all the rank combinations between methods (i.e. colours and numbers). The *y*-axis shows the number of territories (frequency) in which any of the colour–number combinations occurred.

(ICC = 0.27, $P = 0.16$). Similarly, the biomass estimation and SIAR approaches showed a significant agreement for pigeons (ICC = 0.43, $P < 0.05$) and Gulls (ICC = 0.43, $P < 0.05$), but not for Rabbits (ICC = 0.31, $P = 0.11$), passerines (ICC = 0.29, $P = 0.14$) or Squirrels (ICC = 0.29, $P = 0.14$). Lastly, there was poor agreement for estimates of Partridges, both between prey item counts and SIAR (ICC = –0.32, $P = 0.81$) and between biomass estimation and SIAR (ICC = –0.18, $P = 0.71$; Table 2).

Table 2. Intraclass correlation coefficients (ICCs) and *P*-values (*P*) when comparing prey consumption estimates at the territory level between ITEMS and SIAR, or between BIOMASS and SIAR. Results include both the overall intraclass correlation ($n = 516$), and the intraclass correlation done by prey ($n = 43$). Prey categories are European Rabbits (OC), pigeons (CSP), Red-legged Partridges (AR), passerines (PAS), Yellow-legged Gulls (LM) and Eurasian Red Squirrels (SV). Significant *P*-values (< 0.05) are shown in bold type.

	ICCs	*P*
Overall intraclass correlation		
ITEMS vs. SIAR	0.301	0.125
BIOMASS vs. SIAR	0.286	0.139
	ICCs	***P***
Intraclass correlation by prey		
OC – ITEMS vs. SIAR	0.418	**0.042**
CSP – ITEMS vs. SIAR	0.437	**0.033**
AR – ITEMS vs. SIAR	−0.316	0.812
PAS – ITEMS vs. SIAR	0.210	0.224
LM – ITEMS vs. SIAR	0.549	**0.006**
SV – ITEMS vs. SIAR	0.267	0.159
OC – BIOMASS vs. SIAR	0.314	0.113
CSP – BIOMASS vs. SIAR	0.430	**0.036**
AR – BIOMASS vs. SIAR	−0.183	0.706
PAS – BIOMASS vs. SIAR	0.286	0.140
LM – BIOMASS vs. SIAR	0.432	**0.035**
SV – BIOMASS vs. SIAR	0.289	0.137

DISCUSSION

We assessed the diet of Bonelli's Eagle nestlings in Catalonia, which included both marine and terrestrial prey, using conventional pellet analysis and Bayesian isotopic mixing models based on $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$. The pellet analysis revealed that European Rabbits, pigeons, Red-legged Partridges, passerines, Yellow-legged Gulls and Eurasian Red Squirrels were the main prey items, and these were sampled for SIA. Our prey isotopic characterization accounted for the effect of environmental features (Table 1), and allowed reliable use of SIAR to estimate prey consumption at the territory level. Our results show an overall agreement in main prey consumption estimates between the pellet analysis and SIAR both at the population level and, to a lesser extent, at the territory level, where prey consumption was nonetheless similarly ranked by both methods (pellets vs. SIAR), especially in terms of the most- and least-consumed prey. Method comparisons through intraclass correlation for each prey category showed reasonable similarities, except in the case of Partridges. Overall, our results suggest that a combination of pellet analysis and SIA can be a useful way to assess the diet of predator species, and can add important

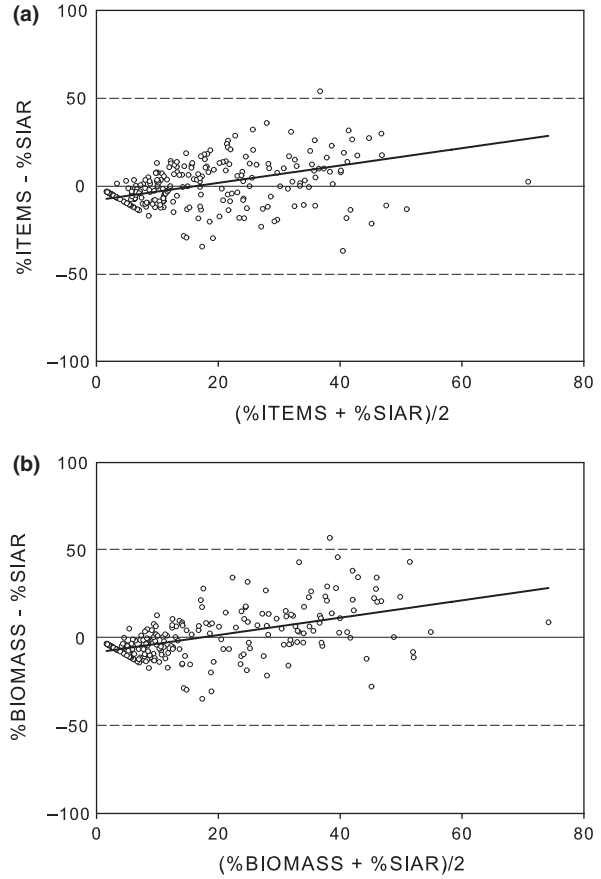

Figure 5. Bland–Altman plots showing the agreement between ITEMS and SIAR (a) or BIOMASS and SIAR (b) in main prey consumption estimates (%) at the territory–year level. The *y*-axis shows the difference in prey consumption estimates between ITEMS and SIAR (a) or BIOMASS and SIAR (b). The *x*-axis shows mean prey consumption estimates from both methods: (ITEMS+SIAR)/2 (a) or (BIOMASS+SIAR)/2 (b). Solid black lines at 0 indicate total method agreement (i.e. both methods estimated the same prey consumption percentage), whereas dashed lines at ± 50 indicate a disagreement in the method's prey estimates higher or lower than 50%. The linear trend between the variables plotted is shown.

insights with the application of isotopic analysis to study raptor food habits.

Conventional and isotopic methods each have advantages and disadvantages. Pellet analysis is non-invasive and allows detailed prey identification. However, it has potential biases related to prey size or digestibility (Votier *et al.* 2003, Marti *et al.* 2007), and may involve great effort in terms of both data collection and analysis of pellet contents. In contrast, SIA generates data about assimilated rather than ingested prey. Furthermore, isotopic analysis has the advantage that it provides diet estimates from the sampled individuals, which is frequently unachievable through conventional dietary methods, for example when several chicks are raised in the same nest. However, the use of isotopic mixing models to estimate prey consumption requires accurate prior information of the species' feeding ecology to select the right prey for tissue analysis, as well as suitable. Moreover, tissue collection for isotopic analysis requires handling of both consumer and prey, and laboratory analyses are more expensive than for conventional diet analyses. Therefore, conventional pellet and isotopic analyses can be considered complementary methods to monitor dietary patterns in territorial birds.

Our dietary results accord with other studies of Bonelli's Eagle in northeast Iberia, where the species takes more pigeons and fewer Rabbits and Partridges than populations in the southern Iberian Peninsula (Moleón *et al.* 2009). Moreover, in our study area, near the northern limit of the species' distribution in western Europe, local environmental conditions are more heterogeneous among territories than in southern populations, and this probably translates into greater dietary differences between territories, with some territorial pairs preying disproportionately on prey species that may be considered secondary or suboptimal elsewhere. For example, we show that Yellow-legged Gulls may constitute an important prey for some territorial pairs located in coastal areas, probably due the high abundance of Gulls in those territories (see also Resano *et al.* 2012).

Despite the importance of a comprehensive isotopic characterization of prey as the basis for SIA, logistical difficulties generally constrain prey sample collection. Therefore, published studies often present low prey sample sizes and rarely consider spatial heterogeneity in prey isotopic values (but see Hebert *et al.* 1999, Ramos *et al.* 2009, Moreno *et al.* 2010). In our study, we obtained large samples of individuals in most prey categories and achieved this across the whole study area, to assess whether individuals differed in their isotopic values due to environmental variation caused by proximity to the sea or habitat variation. For Rabbits and Domestic Pigeons we found that individuals collected in Eagle territories close to the sea showed higher $\delta^{34}S$ than individuals from inland territories (Fig. 2), in accordance with the general trend of higher $\delta^{34}S$ in species inhabiting marine ecosystems (Thode 1991, Deegan & Garritt 1997, Connolly *et al.* 2004). Moreover, Yellow-legged

Gulls, the only marine prey species detected in the diet of Bonelli's Eagle in our study, showed the highest $\delta^{34}S$ values of all analysed prey. This supports the use of $\delta^{34}S$ in distinguishing among the terrestrial and marine components in the diet of predator species foraging in both marine and terrestrial ecosystems (Moreno *et al.* 2010, Ramos *et al.* 2013). Conversely, prey isotopic values did not vary across habitat types, except in the case of Domestic Pigeons from agricultural habitats, which showed significantly higher $\delta^{15}N$ than those from scrubland habitats (Fig. 2). This may be related to the use of nitrate-based fertilizers in agricultural areas (Choi *et al.* 2007) and the tendency of Domestic Pigeons to forage on agricultural crops in the study area (Authors pers. obs.). Based on these results, prey isotopic values included in SIAR for European Rabbits and Domestic Pigeons were selected according to environmental features (i.e. proximity to the sea and habitat type) of Eagle territories (Table 1), thus allowing consideration of spatial heterogeneity in prey isotopic values and increasing model accuracy when estimating Bonelli's Eagle nestling diet. The fact that the isotopic values of nestlings generally lay within the δ-space delineated by main prey categories previously corrected by TEFs (Fig. 1) suggested both that main prey categories were representative of nestling diet, and that prey isotopic values and TEFs were reasonable. Overall, our isotopic characterization of prey highlights the importance of an extensive prey sampling strategy to avoid equivocal interpretations from isotopic prey base values and to resolve mixing models with higher reliability.

There was an overall agreement between pellet analysis (in terms of both items and biomass) and SIAR when estimating prey consumption by Bonelli's Eagles at the population level. Both methods estimated similar means and ranges for prey consumption, and showed that Rabbits and pigeons were consumed more than Partridges, passerines, Gulls and Squirrels (Fig. 3). Our results therefore suggest that both pellet analysis and SIAR are suitable methods to assess the diet of avian predators at the population level (Real 1996, Resano *et al.* 2011). At the territory level, we also found broad agreement in the relative rankings of prey consumption rates; that is, the most-consumed prey as assessed by pellet analysis was the same prey category identified as most-consumed by SIAR, and similarly for the least-consumed prey category (Fig. 4). This result supports the applicability of isotopic mixing models to infer main prey consumption patterns in territorial raptor species. In contrast, the intraclass correlation coefficient showed poor agreement when comparing consumption estimates of all prey categories in the same analysis, probably due to differences in agreement between methods for individual prey categories. For instance, the prey item counts and SIAR showed agreement in their consumption estimates of Rabbits, pigeons and Gulls, and biomass estimation and SIAR did the same for pigeons and Gulls (Table 2). We did not find significant agreement between methods for passerines and Squirrels; however, the ICCs showed certain similarities between method for those prey, especially between the biomass estimation and SIAR. The fact that passerines and Squirrels were the main prey categories with the lowest biomass could make them susceptible to be underestimated by the pellet analysis. Finally, we found poor agreement between methods in consumption estimates for Partridges, although we could not identify any evidence or possible causes to explain this.

Despite an overall agreement between the pellet analysis and SIAR in terms of main prey estimates, noticeable method discrepancies were found in the estimated percentages of some prey, especially at the territory level. This was not related to the origin of pellets (i.e. adults vs. nestlings) because adults rarely leave pellets in the nest (J. Real pers. obs.), but could be related to the fact that pellets and nestling feathers were temporally mismatched. Pellets represented nestling diet during the whole rearing period, whereas the isotopic composition of nestling feathers represented diet during feather growth (i.e. approximately half of the whole rearing period). Nevertheless, this would only affect our results in those cases where nestling diet changed during the second half of the rearing period.

In conclusion, our results support the potential of intrinsic biogeochemical markers (i.e. $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$) to infer the main prey consumption of raptor nestlings by analysing the isotopic composition of their feathers. Moreover, and in accordance with other isotopic studies of predator species, the use of $\delta^{34}S$ could serve to assess the marine prey components in those raptor species foraging on both terrestrial and marine ecosystems (see Chamberlain *et al.* 2005). The use of isotopic mixing models to assess nestling diet would also

allow individual diet estimates, thus offering a valuable approach to investigate the foraging ecology of individuals within a population, its ecological causes and fitness or evolutionary consequences. Nevertheless, the use of isotopic mixing models requires previous information of the species' feeding ecology, usually assessed by conventional diet analysis, a comprehensive prey isotopic characterization and reliable TEF estimates, which are usually available for some model species (e.g. Peregrine Falcon) but may be difficult to obtain for a particular species of interest. Future empirical research will contribute to a deeper understanding of the applicability and potential biases associated with isotopic analyses in avian predators, and we particularly encourage research to evaluate the usefulness of isotopic approaches to study the foraging ecology and its ecological implications in raptor species worldwide.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Table S1. Diet of Bonelli's Eagle nestlings in Catalonia based on pellet analysis (n = 979 pellets analysed).

Table S2. Prey consumption (mean ± sd; %) of main prey categories estimated by each dietary approach: ITEMS, BIOMASS and SIAR.