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USING STABLE ISOTOPES TO DETERMINE DIETARY PATTERNS IN BONELLI'S EAGLE (*AQUILA FASCIATA*) NESTLINGS

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ABSTRACT.—Bonelli's Eagle (*Aquila fasciata*) is one of the most endangered raptor species in Europe due to high adult and subadult mortality rates, habitat loss, and a decrease in populations of its most important prey, European rabbits (*Oryctolagus cuniculus*) and Red-legged Partridges (*Alectoris rufa*). During the breeding season of 2008, we studied the diet of Bonelli's Eagles at 15 breeding territories in Catalonia, northeastern Iberian Peninsula, through a conventional pellet analysis and stable isotope analyses (SIA) of nestlings' feathers. Our objectives were to investigate the diet of Bonelli's Eagle nestlings and to determine whether SIA allowed accurate representation of their dietary patterns. The pellet analysis revealed a broad diet including pigeons (*Columba* spp.; 31.1%), European rabbits (27.9%), ''other birds'' (16.2%), Red-legged Partridges (13.1%), Eurasian red squirrels (*Sciurus vulgaris*; 5.2%), ocellated lizards (*Timon lepidus*; 2.6%), Yellow-legged Gulls (*Larus michahellis*; 2.2%) and ''other mammals'' (1.7%). Diet composition was heterogeneous and varied markedly among nestlings from different breeding territories. We found a significant positive correlation between $\delta^{13}C$ and the frequency of Eurasian red squirrels in the diet, and a significant negative correlation between $\delta^{13}C$ and the frequency of Red-legged Partridges, which are species that occur in forested and open habitats, respectively. The values of $\delta^{15}N$ were not correlated with the consumption of any prey category. However, its wide range of values suggested a global diet with a broad diversity of prey species from at least two different trophic levels. Finally, $\delta^{34}S$ were higher for those nestlings that fed on Yellow-legged Gulls. Our study provided the first isotopic approach to the trophic ecology of Bonelli's Eagle nestlings, and we concluded that $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$ may be useful for assessing nestlings' dietary patterns in terms of main prey consumption and prey trophic level.

KEY WORDS: *Bonelli's Eagle*; *Aquila fasciata*; *Hieraaetus fasciatus*; *diet*; *pellet analysis*; *raptor*; *stable isotopes.*

USO DE ISÓTOPOS ESTABLES PARA DETERMINAR TENDENCIAS TRÓFICAS EN POLLOS DE *AQUILA FASCIATA*

RESUMEN.—El águila *Aquila fasciata* es una de las rapaces más amenazadas de Europa debido a la elevada tasa de mortalidad adulta y preadulta, la degradación y pérdida del hábitat, así como una disminución de sus principales presas como el conejo europeo (*Oryctolagus cuniculus*) o la perdiz roja (*Alectoris rufa*). Durante la temporada de cría de 2008 se estudió la dieta de 15 parejas reproductoras de *A. fasciata* en Catalunya, noreste de la Península Ibérica, a través del análisis convencional de egagrópilas y el análisis de isótopos estables (AIE) en las plumas de los pollos. Nuestros objetivos fueron investigar la dieta de los pollos de *A. fasciata*, así como determinar si el AIE permite representar con exactitud sus patrones tróficos. El análisis de egagrópilas reveló una dieta variada que incluyó palomas (*Columba* spp.; 31.1%), conejo europeo (27.9%), ''otras aves'' (16.2%), perdiz roja (13.1%), la ardilla *Sciurus vulgaris* (5.2%), el lagarto *Timon lepidus* (2.6%), la gaviota *Larus michahellis* (2.2%) y ''otros mamíferos'' (1.7%) como principales categorías de presas. Sin embargo, la composición de la dieta fue heterogénea y se hallaron diversos patrones tróficos entre pollos pertenecientes a diferentes territorios de cría. Asimismo, se halló correlación positiva entre $\delta^{13}C$ y la frecuencia de ardilla roja en la dieta, y negativa entre $\delta^{13}C$ y la frecuencia de perdiz roja, especies presentes en hábitats boscosos y abiertos, respectivamente. No hubo correlación entre $\delta^{15}N$ y el consumo de presas. Sin embargo, su amplio rango de valores sugirió una dieta con diversidad de presas pertenecientes, al menos, a dos niveles tróficos diferentes. Finalmente, $\delta^{34}S$ fue mayor en aquellos pollos que consumieron la gaviota *L. michahellis*. Este estudio aborda por vez primera la ecología trófica en pollos de *A. fascista* a partir del AIE, concluyendo que $\delta^{13}C$, $\delta^{15}N$ y $\delta^{34}S$ son útiles para la evaluación de sus patrones tróficos en términos de consumo de las principales presas y niveles tróficos de las mismas.

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The study of raptors' feeding habits provides meaningful information that can aid the understanding of species' dietary ecology and their trophic relationships at the community level (Jaksic and Delibes 1987, Newton 1998, Marti et al. 2007). The assessment of raptors' dietary patterns may also provide information about distribution, abundance, behavior and the vulnerability of prey species (del Hoyo et al. 1994, Johnsgard 2002). Traditionally, the diets of raptors are described using conventional methodologies that include the analysis of regurgitated pellets, food remains from nests, and stomach contents, as well as the direct observation of prey delivered to nestlings at the nests (Korpimäki and Norrdahl 1991, Salamolard et al. 2000, Katzner et al. 2006, Marti et al. 2007). Of these methods, pellet analysis is the most common approach in the study of raptors' dietary habits, both quantitatively and qualitatively, and has been shown to be an efficient and suitable method for monitoring the diet of several raptor species (Real 1996, Marti et al. 2007). The main advantage of conventional methods is that they frequently enable prey to be identified at the species or taxonomic group level. However, differences in prey sizes, digestion, and consumption patterns may lead to biases such as the over- or underestimation of the proportions of prey items in a predator's diet (Real 1996, Votier et al. 2003, Marti et al. 2007, Sánchez et al. 2008). Moreover, due to the logistical difficulty in sampling regularly over an extended period of time, conventional methods may in fact reflect only short-term dietary habits (Inger and Bearhop 2008).

Over the last two decades, stable isotope analysis (SIA) has become increasingly common in avian trophic ecology as a means of studying foraging strategies and dietary specialization at both individual and population levels (Kelly 2000, Bolnick et al. 2002, Rubenstein and Hobson 2004, Araújo et al. 2009). The use of SIA in dietary studies relies on the fact that different dietary items often have different isotopic values, which are reflected in the tissue of the consumers (Pearson et al. 2003, Becker et al. 2007, Inger and Bearhop 2008). For example, metabolically inert tissues such as feathers preserve the isotopic composition of resources incorporated while growing (Hobson 1999, Bearhop et al. 2002), and the use of SIA in avian trophic ecology has been shown as a powerful means of integrating temporal dietary information, particularly when combined with conventional methods (Inger and Bearhop 2008).

Stable carbon ($^{13}C/^{12}C$, $\delta^{13}C$) and nitrogen ($^{15}N/^{14}N$, $\delta^{15}N$) isotopes are the most frequently used isotopes in the study of trophic relationships and food-web structures at community level (Kelly 2000). The carbon-isotope composition of a consumer enables the carbon sources of the primary production within a food web to be determined (Krouse and Herbert 1988, Crawford et al. 2008). Nitrogen isotopes are useful for diagnosing the species' trophic level position since consumers are typically enriched in ^{15}N by 3–5‰ in proportion to the food they consume (Post 2002, Vanderklift and Ponsard 2003). This finding has been used to provide insights into community-level phenomena such as trophic cascades, the length of food chains, and resource partitioning (Post 2002, Roemer et al. 2002). In addition, the analysis of stable sulphur isotopes ($^{34}S/^{32}S$, $\delta^{34}S$) has been recommended in dietary studies as a means of discriminating between prey from marine and terrestrial ecosystems (Peterson et al. 1985, Peterson and Fry 1987). However, despite the wide applicability of SIA in avian foraging ecology, few isotopic studies have focused on terrestrial top predators such as raptor species (but see Roemer et al. 2002, Dominguez et al. 2003, Caut et al. 2006).

Bonelli's Eagle (*Aquila fasciata*) is a medium-sized raptor distributed from Southeast Asia and the Middle East to the western Mediterranean (del Hoyo et al. 1994). Its European population has declined markedly from the 1970s to the early 1990s (Rocamora 1994, Real 2004) and this raptor is now listed as an endangered species (BirdLife International 2004). In Europe, Bonelli's Eagle occupies Mediterranean mountain ranges and lowlands, and forages mainly in scrublands and dry fields where it predates on a wide variety of species ranging from medium-sized to small mammals (Lagomorpha and Rodentia), birds (Galliformes, Columbiformes, Charadriiformes, Passeriformes, and others) and occasionally reptiles (mainly lizards; Real 1991, Martínez et al. 1994, Iezekiel et al. 2004, Ontiveros et al. 2005, Palma et al. 2006, Moleón et al. 2009a, 2009b). Furthermore, marked dietary differences may exist among territories due to heterogeneity in ecological features such as habitat coverage, prey abundance and distribution, and human pressure (Real et al. 2004). Therefore, Bonelli's Eagle is a suitable model for assessing whether territorial dietary patterns inferred by conventional techniques can also be described using isotopic data. Moreover, the ecological features of some territories have undergone great changes in recent decades (i.e., the expansion of forests as a consequence of land abandonment, an increase in human pressure that results from sprawl, and greater demands for leisure activities), and the number and availability of prey species has been greatly modified. Thus, the ongoing monitoring of diet of Bonelli's Eagle may constitute a good tool to assess the prey on which this species depends during the nestling period, and also help illuminate how habitat changes may affect eagles' foraging habits.

The focus of our study was an analysis of Bonelli's Eagle diet during the breeding season via conventional pellet analysis and an evaluation of the usefulness of SIA for assessing nestlings' dietary patterns. The specific aims of this study were: (1) to assess the diet of Bonelli's Eagle nestlings from different breeding territories using conventional pellet analysis; (2) to describe stable isotope values ($\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$) in nestlings' feathers; (3) to assess isotopic data in siblings as indicators of diet similarity; and (4) to test whether isotopic data from nestlings were related to their prey consumption as described by the pellet analysis.

METHODS

Study Area. During 2008, we studied 15 territorial breeding pairs of Bonelli's Eagle in Catalonia (northeastern Spain; 01°32′E, 41°20′N). Sampled territories were a subset of known territories for the species in Catalonia. All sampled nests were located on cliffs, and environmental features in breeding territories varied but were representative of Mediterranean habitats, and included scrublands (*Quercus coccifera, Thymus vulgaris, Pistacia lentiscus* and *Rosmarinus officinalis*), woodland patches (mainly *Quercus ilex* and *Pinus* spp.), nonirrigated cropland and built-up areas (Bosch et al. 2010). The mean altitude of nesting areas ranged from 176 to 753 m asl, with mean annual rainfall ranging from 450 to 800 mm.

Data Collection. Each breeding territory was monitored between January and July. We checked each territory using a spotting scope (20–60×) between January and early March to assess territorial occupancy and breeding activity (i.e., displays, nest material transfer, copulation, and incubation behavior). In late March and April, we checked nests again, using a spotting scope, to detect the presence, number, and approximate age of nestlings. The age of nestlings was estimated by the development of feathers and by calculating from the laying date (Real 1991, Gil-Sánchez 2000). After nestlings were approximately 37 d old, climbers accessed nests to collect 3–4 feathers from the back of each nestling for the SIA, assuming that isotopic data from nestlings' feathers were representative of the whole nestling period. At the same time, pellets were collected from the nest for the conventional diet analysis. Finally, approximately 2 wk after the nestlings had fledged, nests were visited again for a second retrieval of pellets. Therefore, we assumed that our conventional diet study based on pellet analysis was representative of nestlings' diet during their entire nestling period.

Conventional Diet Study and Statistical Procedures. The conventional diet study was based on pellet analysis. Pellets were individually analyzed and each prey species identified in a pellet was counted as one individual (Real 1996, Gil-Sánchez et al. 2004). Pellets were visually examined and their contents (i.e., feathers, bones, hair, nails, and scales) were compared with prey items from our own reference collection. For some remains, such as feathers, we also used a 4× magnifying glass and consulted specialized guides for the identification of macro- and microscopic remains (Brom 1986). Prey were identified to species level whenever possible.

Prey items were grouped into eight different taxonomic categories: European rabbits (*Oryctolagus cuniculus*), Eurasian red squirrels (*Sciurus vulgaris*), "other mammals," pigeons (*Columba* spp.), Red-legged Partridges (*Alectoris rufa*), Yellow-legged Gulls (*Larus michahellis*), "other birds" (mainly Corvidae and Turdidae) and ocellated lizards (*Timon lepidus*). Diet data were analyzed at the territory level by comparing the frequency (%) of items in each taxonomic group relative to the total number of prey items (Palma et al. 2006, Moleón et al. 2009b). To assess the dietary patterns of nestlings at the territory level, we performed a principal component analysis (PCA) of prey frequency consumption using the varimax rotation, which keeps the rotated components orthogonal to or uncorrelated with each other after rotation (Quinn and Keough 2002). Additionally, we performed Spearman rank correlation tests (r_s) for all taxonomic prey consumption at the territory level.

Stable Isotope Analysis and Statistical Procedures. Nestling feathers were frozen until they were cleaned in a solution of NaOH (0.25 M; Bearhop et al. 2002, Ramos et al. 2009) and oven-dried at 40°C for 24 hr. Lipids were not washed off the feathers as they were shown to have negligible effects on the isotope ratios (Mizutani et al. 1992). To homogenize samples, feathers were ground into an extremely fine powder using an impactor mill (6750 Freezer/Mill, Spex Certiprep, Metuchen, New Jersey, U.S.A.) operating at the temperature of liquid nitrogen. 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 recipients and crimped for combustion. Isotopic analyses were conducted using elemental analysis-isotope ratio mass spectrometry (EA-IRMS) using a Flash 1112 (for C and N)/1108 (for S) elemental analyzer coupled to a Delta C isotope ratio mass spectrometer via a CONFLOIII interface (Thermo Fisher Scientific, Bremen, Germany). Analyses were performed at the Scientific Technical Services of the University of Barcelona.

Stable isotope ratios are expressed conventionally as parts per thousand (‰), according to the following equation: $\delta X = [(Rsample/Rstandard) - 1] \times 1000$, 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 $^{34}S/^{32}S$. Samples were referenced against international standards: Pee Dee Belemnite (VPDB) for ^{13}C, atmospheric nitrogen (AIR) for ^{15}N and Canyon Diablo Troilite (CDT) for ^{34}S. The measurement precisions for $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$ were $\leq$0.15‰, $\leq$0.25‰ and $\leq$0.4‰, respectively.

Arithmetic mean values (±SD) for $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$ were calculated for all nestlings. Because we expected Bonelli's Eagle nestlings raised in the same nest to have similar prey intake, we tested whether the isotopic values from siblings hatched in the same nest were more similar to each other than to isotopic values from a random sample of nestlings from the studied population. First, we applied a Spearman rank correlation test that only considered those territories where two nestlings were born ($n = 9$), and we then performed a randomization test to assess whether isotopic similarities between siblings differed from the expected random distribution. To do so, we obtained two samples of nine individuals extracted at random from the pool of the studied population ($n = 24$ nestlings) and compared their isotopic values with a Spearman rank correlation. This step was repeated 10 000 times and the resulting correlation coefficients were recorded. Next, we calculated the proportion of randomized coefficients that were recorded as equal to or larger than the observed correlation coefficient in siblings. This proportion, our estimated *P*-value (*P*), was then used to accept or reject the assertion that isotopic values were more similar between siblings than the expected random distribution.

Finally, we analyzed whether isotopic data from nestlings were related to their diet as estimated by the pellet analysis. To do so, we performed a Spearman rank correlation test between $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$ from nestlings from each breeding pair and nestlings' prey consumption as described by the pellet analysis. Nestlings from the same nest/territory were considered a single statistical observation and the isotopic values ($\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$) for each breeding territory were estimated using the means of the two siblings.

Statistical analyses were conducted using R software (R Development Core Team 2007) and SPSS 15.0 (SPSS, Chicago, Illinois, U.S.A.).

RESULTS

Conventional Diet. We identified 542 prey items in the 241 pellets analyzed (Table 1). In all, 62.6% of prey items were birds, 34.8% were mammals, and 2.6% were reptiles. The main prey items consumed were pigeons (31.1%), a category that included Rock Pigeon (*Columba livia*), Common Wood-pigeon (*Columba palumbus*), and Stock Dove (*Columba oenas*), followed by European rabbits (27.9%), ''other birds'' (16.2%), Red-legged Partridges (13.1%), Eurasian red squirrels (5.2%), ocellated lizards (2.6%), Yellow-legged Gulls (2.2%), and ''other mammals'' (1.7%; Fig. 1).

The PCA revealed marked dietary patterns between nestlings from different territories (Table 2 and Fig. 2). The first two components accounted for 64.6% of total diet variance. The first component, which accounted for 40.3% of diet variance, discriminated between nestlings with a high consumption of pigeons as opposed to others whose diet included more Red-legged Partridges, ocellated lizards, Yellow-legged Gulls and ''other mammals.'' The second component explained an additional 24.3% of diet variance and discriminated between greater amounts of European rabbits as opposed to ''other birds.'' Indeed, Spearman rank correlations between taxonomic prey consumption of nestlings at the territory level showed that intake of pigeons was negatively correlated with that of Red-legged Partridges ($r_s = -0.547, P < 0.05$), ocellated lizards ($r_s = -0.685, P < 0.005$), and Yellow-legged Gulls ($r_s = -0.465$, $P < 0.1$). Accordingly, there was also a significant negative correlation between consumption of European rabbits and ''other birds'' ($r_s = -0.526, P < 0.05$).

Table 1. Diet of Bonelli's Eagle nestlings during the breeding season, shown as the number of prey items and their frequencies (%), based on pellet analyses.

PREY SPECIES	NUMBER OF ITEMS	FREQUENCY (%)
Mammals		
European rabbit (*Oryctolagus cuniculus*)	151	27.9
Eurasian red squirrel (*Sciurus vulgaris*)	28	5.2
Undetermined mammal	9	1.7
Total mammals	**188**	**34.8**
Birds		
Northern Goshawk (*Accipiter gentilis*)	4	0.7
European Honey-buzzard (*Pernis apivorus*)	1	0.2
Red-legged Partridge (*Alectoris rufa*)	71	13.1
Common Pheasant (*Phasianus colchicus*)	1	0.2
Galliforms (*Galliform* spp.)	6	1.1
Rock Pigeon (*Columba livia*)	12	2.2
Common Wood-pigeon (*Columba palumbus*)	62	11.3
Stock Dove (*Columba oenas*)	3	0.6
Pigeons (*Columba* spp.)	92	17.0
Eurasian Jay (*Garrulus glandarius*)	12	2.2
Black-billed Magpie (*Pica pica*)	3	0.6
Eurasian Blackbird (*Turdus merula*)	7	1.3
Turdus sp.	1	0.2
Yellow-legged Gull (*Larus michahellis*)	12	2.2
Common Cuckoo (*Cuculus canorus*)	2	0.4
Eurasian Green Woodpecker (*Picus viridis*)	1	0.2
Amazona sp.	4	0.7
Anas sp.	1	0.2
Undetermined bird	45	8.2
Total birds	**340**	**62.6**
Reptiles		
Ocellated lizard (*Timon lepidus*)	14	2.6

Stable Isotopes. The arithmetic mean isotopic values (±SD) for the 24 nestlings were −22.10‰ (±1.03) for δ^{13}C, 6.44‰ (±1.27) for δ^{15}N, and 4.30‰ (±1.43) for δ^{34}S. Isotopic values of individuals from all the different territories showed broad ranges for the three elements (Fig. 3). However, we found that those nestlings hatched and reared in the same nest/territory had significant positive correlations for δ^{13}C ($r_s = 0.93$, $P < 0.001$), δ^{15}N ($r_s = 0.98$, $P < 0.001$), and δ^{34}S ($r_s = 0.95$, $P < 0.001$), and that these correlation values were in all cases significantly higher than expected by a random distribution ($P < 0.001$).

Conventional Diet vs. Stable Isotopes. We found a significant positive correlation between δ^{13}C in nestlings and the frequency of Eurasian red squirrels in their diet ($r_s = 0.565$, $P < 0.05$), as well as a significant negative correlation between δ^{13}C and the frequency of Red-legged Partridges ($r_s = -0.688$, $P \leq 0.005$) (Table 3). Despite not correlating with any particular prey item, high levels of δ^{34}S were found in the nestlings hatched in the two territories where Yellow-legged Gulls were consumed.

DISCUSSION

The diet of Bonelli's Eagle in Catalonia during the nestling period primarily included medium-sized birds such as pigeons and Red-legged Partridges, mammals including European rabbits and Eurasian red squirrels, as well as a variety of less frequently consumed birds (Yellow-legged Gulls, Corvidae, and Turdidae) and a single reptile (ocellated lizard). This diet composition agreed with the general patterns found in other western European populations, where, overall, rabbits, pigeons, partridges, and corvids were the most frequently eaten prey (Real 1991, Martínez et al. 1994, Iezekiel et al. 2004, Ontiveros et al. 2005, Palma et al. 2006, Mo-

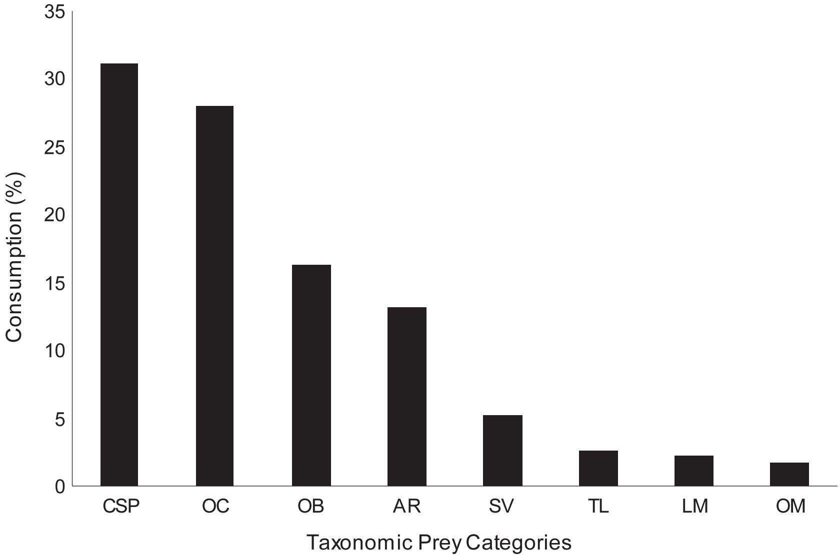

Figure 1. Prey consumption (%) by Bonelli's Eagle nestlings, Catalonia, Spain, as determined by pellet analysis. Taxonomic categories are ordered from greatest to lowest importance in diet: CSP (pigeon [*Columba* spp.]), OC (European rabbit [*O. cuniculus*]), OB (''other birds''), AR (Red-legged Partridge [*A. rufa*]), SV (Eurasian red squirrel [*S. vulgaris*]), TL (ocellated lizard [*T. lepidus*]), LM (Yellow-legged Gull [*L. michahellis*]) and OM (''other mammals'').

león et al. 2009b), and was particularly similar to diets described for the Mediterranean coastal strip of Spain and France, where rabbits are more scarce and the consumption of pigeons and ''other birds'' is greater (Moleón et al. 2009b).

In our study, the PCA suggested that the consumption of the two dominant prey types (pigeons and rabbits) determined the intake of other prey species. For example, those territories with low consumption of pigeons had greater intake of alterna-

Table 2. Prey category consumption (%) of nestlings at the territory level, based on pellet analyses. CSP (pigeon [*Columba* spp.]), OC (European rabbit [*O. cuniculus*]), OB (''other birds''), AR (Red-legged Partridge [*A. rufa*]), SV (Eurasian red squirrel [*S. vulgaris*]), TL (ocellated lizard [*T. lepidus*]), LM (Yellow-legged Gull [*L. michahellis*]) and OM (''other mammals'').

TERR	CSP	OC	OB	AR	SV	TL	LM	OM
1	33.3	33.3	13.3	0	20.1	0	0	0
2	25.6	33.3	20.5	7.7	7.7	2.6	0	2.6
3	47.4	10.5	23.7	15.8	0	0	0	2.6
4	3.8	34.2	6.3	26.6	0	10.1	12.7	6.3
5	45.7	8.6	31.3	2.9	8.6	2.9	0	0
6	66.7	33.3	0	0	0	0	0	0
7	29	38.7	6.5	25.8	0	0	0	0
8	54.9	13.7	9.8	7.8	11.8	0	0	2
9	29	38.8	16.1	16.1	0	0	0	0
10	35.7	35.7	14.4	7.1	7.1	0	0	0
11	13.6	18.2	31.8	22.7	4.6	9.1	0	0
12	23.6	20.6	35.3	5.9	8.8	2.9	0	2.9
13	37.1	44.4	14.8	3.7	0	0	0	0
14	26.5	20.6	23.5	11.8	8.8	2.9	5.9	0
15	31.4	37.2	5.7	20	5.7	0	0	0

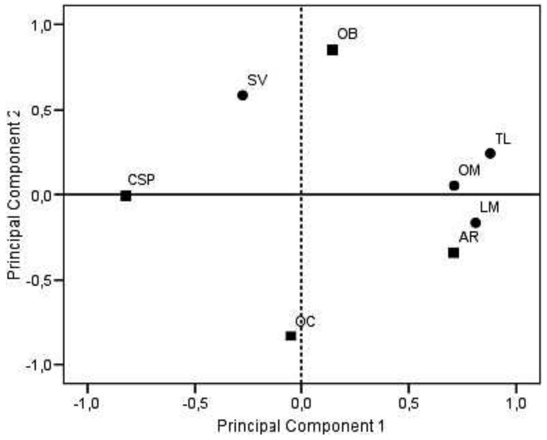

Figure 2. Principal component analysis of taxonomic prey category consumption at territory level. Components 1 and 2 (X-axis and Y-axis, respectively) provide information regarding the rotated and dimensionally reduced diet data. CSP (pigeon [*Columba* spp.]), OC (European rabbit [*O. cuniculus*]), OB ("other birds"), AR (Red-legged Partridge [*A. rufa*]), SV (Eurasian red squirrel [*S. vulgaris*]), TL (ocellated lizard [*T. lepidus*]), LM (Yellow-legged Gull [*L. michahellis*]) and OM ("other mammals"). Solid black squares represent frequently consumed prey and solid black circles represent less frequently consumed prey.

tive prey species such as partridges or, less frequently, Yellow-legged Gulls, ocellated lizard, and small mammals. Similarly, in those territories where rabbits were not frequently consumed, other medium-sized bird species were more important. Variations in diet of Bonelli's Eagle in western Europe seem to be a function of spatio-temporal variation in the abundance of rabbits and the presence of alternative prey species, in conjunction with territorial environmental features (Moleón et al. 2009b). Consequently, the different dietary patterns found in our study at the territory level were likely influenced by the high heterogeneity in ecological features within territories, including habitat, and prey density and distribution.

Stable isotope signatures from nestlings exhibited broad ranges for the three elements we measured ($\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$), a finding that agreed with the high diversity of taxonomic prey items revealed by the conventional pellet analysis. Consumers incorporate carbon into their tissues with an increase of around 1‰ in ^{13}C relative to their food (Kelly 2000) and so the wide range of $\delta^{13}C$ observed in our study (3.76‰) is probably due to a heterogeneous intake of prey species with different carbon

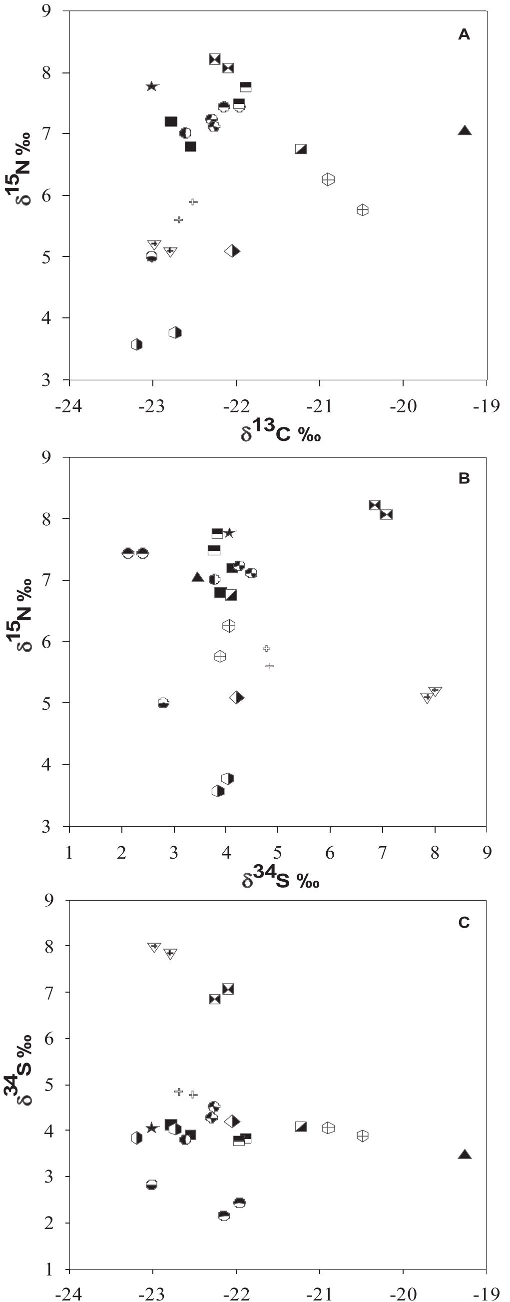

Figure 3. Isotopic values ($\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$) of Bonelli's Eagle nestlings. Different symbols are associated with different territories (n = 15); nine territories had two nestlings. (a) $\delta^{15}N$ vs. $\delta^{13}C$, (b) $\delta^{15}N$ vs. $\delta^{34}S$ and (c) $\delta^{34}S$ vs. $\delta^{13}C$.

Table 3. Spearman correlation values (r_s) for correlations between diet of nestlings as determined by pellet analysis at the territory level and nestlings' isotopic values. CSP (pigeon [*Columba* spp.]), OC (European rabbit [*O. cuniculus*]), OB (''other birds''), AR (Red-legged Partridge [*A. rufa*]), SV (Eurasian red squirrel [*S. vulgaris*]), TL (ocellated lizard [*T. lepidus*]), LM (Yellow-legged Gull [*L. michahellis*]) and OM (''other mammals''). Significant correlations ($P < 0.05$) are shown in bold type.

	CSP	OC	OB	AR	SV	TL	LM	OM
$\delta^{13}C$	0.411	−0.197	0.057	**−0.688**	**0.565**	−0.258	−0.199	−0.183
P-value	0.128	0.480	0.840	**0.005**	**0.028**	0.353	0.477	0.514
$\delta^{15}N$	0.025	−0.228	0.350	0.091	0.347	−0.036	−0.042	−0.441
P-value	0.930	0.414	0.201	0.747	0.205	0.898	0.881	0.100
$\delta^{34}S$	0.021	0.196	0.057	0.039	−0.338	0.060	0.157	−0.460
P-value	0.940	0.485	0.840	0.889	0.218	0.831	0.576	0.085

isotopic signatures (Gu et al. 1997). Additionally, we found a significant positive correlation between $\delta^{13}C$ and the frequency of Eurasian red squirrels in nestlings' diet, as well as a significant negative correlation between $\delta^{13}C$ and the frequency of Red-legged Partridges. Interestingly, abundances of these two prey species at territory level are dependent on habitat types, with squirrels more common in forested territories and partridges more abundant in open habitats in our study area (Real et al. 1995, Mañosa 2004); these associations suggest that the analysis of $\delta^{13}C$ may be a good indicator of prey consumption and habitat features at the territory level. In the case of nitrogen, consumers are typically enriched in ^{15}N by 3–5‰ relative to their prey (Post 2002, Vanderklift and Ponsard 2003), a fact that allows the trophic level position of the prey species to be assessed (Kelly 2000). In our study, $\delta^{15}N$ ranged from 3.57 to 8.21‰, which suggested that the total diet within our study sample included prey species from at least two different trophic levels. This was supported by the wide range of prey species detected by the conventional pellet analysis, including herbivores (rabbits), granivores (pigeons), secondary consumers (thrushes and Corvidae), and even potential scavengers (Yellow-legged Gulls). Finally, the use of $\delta^{34}S$ in dietary studies has been recommended as a means of distinguishing between terrestrial and marine prey species (Peterson et al. 1985, Moreno et al. 2009). In our study, higher signatures of $\delta^{34}S$ were found at two territories where Yellow-legged Gulls were consumed, and that species was the only marine prey species identified in the pellet analysis. Accordingly, $\delta^{34}S$ signatures of this gull species from the same study area (Ramos et al. 2009) showed similar signatures to those found in Bonelli's Eagle nestlings that consumed it. The lack of significant correlation between $\delta^{34}S$ and the consumption of Yellow-legged Gulls probably resulted from the fact that it was consumed at only 2 of 15 territories.

Our interpretation of the SIA based on the diet composition of Bonelli's Eagle nestlings may be potentially constrained by a number of biases. A basic assumption when using SIA in the assessment of animal diets is that the main prey species have different isotopic composition (Bearhop et al. 2004, Matthews and Mazumder 2004). However, we did not analyze isotopic composition of prey species and instead used indirect evidence to evaluate the suitability of SIA as a means of inferring diet. First, the $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$ of nestlings hatched and raised in the same nest were more similar than would be randomly expected. Given that Bonelli's Eagle nestlings share prey items (Real 1996), our results indicated that the isotopic signatures of nestlings were related to the prey consumed (see also Angerbjörn et al. 1994, Gu et al. 1997, Araújo et al. 2009). Second, we tested whether $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$ were correlated with prey consumption. In fact, we found significant correlations between $\delta^{13}C$ and two prey species, as well as other dietary patterns for $\delta^{15}N$ and $\delta^{34}S$ (above).

In recent decades, the use of stable isotopes in avian foraging studies has been increasingly used as a robust tool for providing long-term information on birds' foraging habits and degree of dietary specialization at both the individual and population level (Kelly 2000, Bolnick et al. 2002, Rubenstein and Hobson 2004, Inger and Bearhop 2008, Araújo et al. 2009). However, few isotopic studies have focused on raptors' dietary habits (but see Roemer et al. 2002, Dominguez et al. 2003, Caut et al. 2006), so the advantages of SIA in studies of raptors' trophic ecology are sometimes underestimated. Our study provided the first reference values for isotopic signatures in Bonelli's Eagle nestlings. One advantage of isotopic analyses is that they may overcome some of the biases traditionally associated with conventional procedures. For example, isotopic data from nestlings' feathers are representative of the nestlings' diet over the entire period of tissue development (Inger and Bearhop 2008), whereas pellets may be representative of a shorter period if they are not collected regularly. Moreover, isotopic data inform about prey digested and absorbed, and may overcome the over- or underrepresentation of certain prey items associated with conventional diet analyses (Inger and Bearhop 2008). In terms of effort, the pellet analysis is more time-consuming than isotopic analysis. SIA may also allow assessment of individual's diets, as, for example, when comparing the diet between siblings or between parents and nestlings. Finally, temporal changes or spatial heterogeneity in diet composition can be addressed with SIA (Bearhop et al. 2001, Rubenstein and Hobson 2004, Chiaradia et al. 2010); by analyzing the isotopic composition of nestlings' feathers, we may be able to monitor temporal variations in prey abundance at the territory level. The major disadvantage of SIA in dietary studies where we do not know the isotopic prey signatures is that we cannot distinguish individual prey species in the predators' diet.

Mediterranean landscapes have undergone important changes in terms of human activity and the extent of different types of land use (Meeus 1993, Butet et al. 2010), and such changes have influenced the distribution and abundance of Bonelli's Eagle prey and hence the conservation of this raptor species (Ontiveros et al. 2005, Moleón et al. 2009b). In our study, SIA proved useful for monitoring nestling Bonelli's Eagles' diets, which may reflect the abundance and distribution of prey at the territory level. Thus, the implementation of SIA on a regular basis at the territory level may be a valuable tool for monitoring not only the biological relationship between Bonelli's Eagle and its prey, but also temporal changes in Mediterranean habitats and ecosystems. Future isotopic analyses will provide further insights and a deeper understanding of the trophic ecology of Bonelli's Eagles.

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