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An exploration of isotopic variability in feathers and claws of Lesser Kestrel *Falco naumanni* chicks from southern Sicily

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Abstract – Stable isotopes are nowadays commonly used in the study of many key features of avian ecology. However, the adequate choice of what isotopic ratio to consider and what tissues to sample for assessing specific questions may be tricky. Here, we explored the variation in a suite of stable isotope ratios ($\delta^{13}C$, $\delta^{15}N$, $\delta^{34}S$, $\delta^{2}H$, $\delta^{18}O$) in the feathers and claws of chicks of Lesser Kestrels *Falco naumanni* from south-eastern Sicily (Italy) sampled throughout colonies of the same population but surrounded by different habitats. Our aims are to provide an insight into the isotopic ecology of Lesser Kestrel and to provide methodological indications for future studies. Specifically, we tested whether stable isotope ratios (i) were consistent between feathers and claws; (ii) differed within and between colonies; (iii) reflected differences in surrounding habitats. We found that all isotope ratios significantly differed between claws and feathers. Hierarchical cluster analyses revealed a high consistency of stable isotope ratios for all the elements between siblings and nests within the same colony. Significant differences in stable isotope ratios emerged among colonies and were associated to differences in the surrounding habitat. The isotope approach has great potential in the study of lesser kestrel ecology, and our results suggest which element should be selected to approach a set of different ecological questions.

Key-words: breeding quarters, claws, feathers, foraging, habitat, colonies, stable isotopes.

INTRODUCTION

The analysis of stable isotopes has grown in relevance in the study of avian ecology, since the ground-breaking papers by Hobson & Clark (1992a, 1992b) on avian diets and by Marra *et al.* (1998) on migratory connectivity and carry-over effects by analysing the isotope composition of different bird tissues. Furthermore, the use of stable isotopes and protocols in avian ecology has strongly improved, so that a wide range of chemical elements are currently used to decipher a vast set of ecological questions (Hobson 2011). A deep *a priori* knowledge of the study system is strongly advised for a better interpretation of the isotope data (Boecklen *et al.* 2011, Hobson 2011). This basic knowledge can be achieved by field-based exploratory studies dedicated to a specific study system that should ideally explore variations in isotope ratios across different groups of individuals and across different tissues of the target species.

A crucial aspect in the design of isotope research is the choice of the tissue to be sampled, because different tissues from a single individual might renew at different points of the life-cycle, possibly carrying a geographic-signalling isotope trace (Hobson 2011, Evans *et al.* 2012). This is of special interest in migratory birds, whose feathers might inform about breeding or wintering areas of the previous year depending on the species-specific moult strategy, whereas claws can carry information about where the birds have been feeding several weeks up to some months prior to sampling depending on their growth rate (Rolshausen *et al.* 2010, Hahn *et al.* 2014, Morganti *et al.* 2015). Body tissues also differ in their turnover rates, which might cause basal differences in the observed isotope ratios, as found by Hobson & Bairlein (2003) in a comparison between

blood *vs.* feathers, and by Evans *et al.* (2012) and Morganti *et al.* (2015) for claws *vs.* feathers. Thus, tissues sampled at the same time-point on the same bird can be informative, but can also confound results if not correctly interpreted.

Given the outstanding relevance of food quality on the survival probability of chicks and thus, on the dynamic and persistence of populations (Newton 1998), it is of pivotal interest to study how foraging areas vary within a single population and which factors affect food supply. In studies of animal foraging ecology and food habits, the isotope approach is more informative than the traditional analysis of pellets, because it has the potential to disclose which food sources are actually metabolised, despite their abundance with respect to other ingested foods. In contrast, pellet analysis can only estimate the quantities and proportion of ingested food, despite their real metabolic value (Hobson *et al.* 1992a, 1992b, Resano-Mayor *et al.* 2014). In the context of colonial birds, according to the Information Centre Hypothesis (ICH, Danchin & Wagner 1997, Serrano *et al.* 2004, Calabuig *et al.* 2008), individuals from the same colony rely on the same feeding areas and therefore, have similar diets. However, individual diet specialisation might arise even in a colonial context (Bolnick *et al.* 2003, Bearhop *et al.* 2006), and an isotope approach has the potential to reveal this behaviour. The consistency of the feeding behaviour within a colony also relates to the importance of the habitat quality in the immediate vicinity of the colony sites. To disclose whether all birds feed chicks with food obtained from the same area and whether this area is a limited buffer around the colony might have crucial conservation implications. Such hypotheses could be easily explored by analysing whether the isotope ratios of siblings are the same as those in other nests, whether chicks from the same colony have similar isotope ratios to those of distant colonies, and whether chicks from distant colonies surrounded by analogous habitats have analogous isotope ratios. Stable isotope studies on colonial birds are still scarce, and those that use raptors as model species are even rarer (Hobson & Koheler 2015, but see Hobson *et al.* 2009, Resano-Mayor *et al.* 2014).

In this study, we preliminarily addressed some of the above questions by studying the Lesser Kestrel *Falco naumanni*, a social raptor (Campobello *et al.* 2012, 2015) of small size (<150 g), whose western European populations overwinter in the Sahel belt region in Africa (Ferguson-Lees & Christie 2001). During reproduction, Lesser Kestrels inhabit colonies of up to 40 pairs (Serrano *et al.* 2004), generally in open ecosystems that are transformed by agriculture, and form networks of nearby colonies, as is the case in Southeastern Sicily (Italy) (Sarà 2010, Campobello *et al.* 2012, 2015). The diet of the Lesser Kestrel during breeding (Pérez-Granados 2010, Rodríguez *et al.* 2010, Catry *et al.* 2016) mainly consists of *Orthoptera* and *Coleoptera*. The conservation status of the Lesser Kestrel was critical in the Palaearctic until few years ago (BirdLife International 2004), as it was dependent on habitat quality and management, which depleted the quality and quantity of food (Tella *et al.* 1998, Rodríguez *et al.* 2006). Although the Lesser Kestrel has only recently been downgraded from Vulnerable to a species of Least Concern (IUCN categories), population trends are highly variable across the Palaearctic range (Iñigo & Barov 2011) and the species might still be declining locally.

In addition to the strict dependence of its conservation status on food quality, the migratory and social behaviour of the Lesser Kestrel make the stable isotope approach ideal to pose a series of ecological questions about the species. In this study, we considered the stable isotope ratios that have been used in ecology to date, to gather information on both the diet ($\delta^{34}S$, $\delta^{15}N$, $\delta^{13}C$) as well as on the geographical location ($\delta^{2}H$, $\delta^{18}O$) of animals (Hobson 2011, García-Peréz & Hobson 2014, Bontempo *et al.* 2014). $\delta^{34}S$, $\delta^{15}N$, $\delta^{13}C$ are strongly related to habitat use and environmental variables as well as dependent on trophic specialization of the studied animal (Minagawa & Wada 1984, Mizutani *et al.* 1992, Caccamise *et al.* 2000, McCutchan *et al.* 2003, Hobson 2003, 2005, Resano-Mayor *et al.* 2014), thus the ideal candidate elements to study diet specialization. In parallel, relationships between $\delta^{2}H$ and $\delta^{18}O$ could provide important additional information on source environmental waters, diets, and climatic conditions during and prior to growth of the tissues (Hobson & Kohler 2015). Furthermore, given their predominantly insectivorous diet, we explored the eventuality that isotopic signature of Lesser Kestrels could be comparable to those of species such as Passerines that occupy lower trophic levels. This may have major implications because in case of a compatibility of the signal found in Lesser Kestrel with those observed in passerines, this open the possibility to use published isoscapes for Europe to geographically assign origin of specimens collected out of the breeding season (i.e. in wintering quarters).

In this study, we assessed whether isotope ratios:

i) differ between feathers and claws grown at the same time;
ii) are congruent with the social organisation of the Lesser Kestrel;
iii) reflect differences in the habitat surrounding the colonies.

METHODS

Study area and sample collection

We collected feathers and claws growing at the same time in Lesser Kestrel chicks from five different colonies within the same breeding area on the Gela Plain (474 km^2, 37°15'N, 14°35'E) in south-eastern Sicily. The plain has a mean annual rainfall of 350 mm and a mean altitude of 160.3 ± 14.27 m a.s.l.

The environment is characterised by a mosaic of pseudo-steppes dominated by artichoke (*Cynara* spp.) fields, wheat and legume cultivation (Triolo *et al.* 2011). We collected feathers and claws of Lesser Kestrel chicks that were found freshly dead in nests during a nest survey on 22–23 June 2014. In total, we sampled 17 chicks belonging to five colonies and seven nests. Specifically, we sampled both claws and feathers (from the mantle area) from six chicks, only feathers from seven chicks and, in four cases of chicks still with no feathers, we sampled only claws. Lesser Kestrel chicks are born with no feathers and develop the whole plumage within a few weeks; therefore, isotope ratios found in growing juvenile feathers should reflect the food received during the nestling phase. Within the same time period, the claws of the chicks also grow and therefore, differences observed in the isotope traces between feathers and claws should only reflect structural differences between the two tissues or the fact that the allocation of nutrients during the development of the two tissues occurs via different metabolic pathways (Bearhop *et al.* 2003).

For each colony and habitat type we had at least a complete nest sampled. The habitat types surrounding the sampled colonies were classified within a circle of 1-km radius around each colony, a buffer that was established because over 75% of feeding attempts of the Lesser Kestrel during reproduction occur within this limit (Bonal & Aparicio 2008). This radius was recently confirmed by movement analysis of satellite-tagged birds from the same study colonies (Bondì S. 2016). Habitat type was established according to Di Maggio *et al.* (2016), who defined land use based on CORINE Land Cover (CLC, EEA, 2000) and reduced the original habitat types to three main groups by Principal Component Analysis. These groups were: (1) dry-grasslands and other semi-natural vegetation (hereafter *Grassland*); (2) non-irrigated arable land (hereafter *Arable*) and (3) semi-permanent irrigated arable land (indicating mainly artichoke cultivation, hereafter *Artichoke*). Based on these criteria, our colonies (ID) Torre Vecchia (1) and Monteleone (2) were classified as 'arable', Giaurone (3) and San Gregorio (5) as 'artichoke', and Settefarine (4) as 'grassland'.

Stable isotope analyses

Feathers and claws were cleaned in a solvent mixture (diethyl ether-methanol 2:1) and were then oven-dried at 60°C and were cut into small pieces with surgical scissors (Bontempo *et al.* 2014). The samples were weighed on a microbalance (CP2P, Sartorius AG, Goettingen, Germany); about 0.30 mg was placed in tin capsules for $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$ analysis, and about 0.20 mg of each sample was placed in silver capsules for $\delta^{2}H$ and $\delta^{18}O$ analysis. Each sample was weighed and analysed three times for $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$, and two times for $\delta^{2}H$ and $\delta^{18}O$ analysis and determination.

The $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$ isotopes were determined in one run by an isotope ratio mass spectrometer (Vario Isotope Cube, Elementar Analysen systeme GmbH, Germany) and the $\delta^{2}H$ and $\delta^{18}O$ isotopes were determined in one run using an isotope ratio mass spectrometer equipped with a TC/EA pyrolyser (Delta Plus XP – ThermoFinnigan, Bremen, Germany). The isotope ratios were expressed in δ notation according to the following formula:

$$\delta = (R_{sample} - R_{standard})/R_{standard} \text{ (Coplen 2011)}$$

where R_{sample} is the isotope ratio measured for the sample and $R_{standard}$ is the isotope ratio of the international standard. The international standards were V-PDB (Vienna-Pee Dee Belemnite) for $\delta^{13}C$, Air for $\delta^{15}N$, V-CDT for $\delta^{34}S$ (Vienna – Canyon Diablo Troilite) and V-SMOW (Vienna – Standard Mean Ocean Water) for $\delta^{2}H$ and $\delta^{18}O$. The isotope ratios were calculated against working in-house standards (a protein for $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$; keratins for $\delta^{2}H$ and $\delta^{18}O$), which were themselves calibrated against international reference materials: hair USGS 42 and USGS 43 for $^{13}C/^{12}C$, $^{15}N/^{14}N$ and $^{34}S/^{32}S$ and benzoic acid IAEA-601 for $^{18}O/^{16}O$. The $\delta^{2}H$ and $\delta^{18}O$ values were calculated against CBS (Caribou Hoof Standard $\delta^{2}H$ = -197.0 ± 1.8‰ and $\delta^{18}O$ = +3.8 ± 0.3‰) and KHS (Kudu Horn Standard, $\delta^{2}H$ = -54.1 ± 0.6‰ and $\delta^{18}O$ = +20.3 ± 0.3‰) through the creation of a linear equation (Bontempo *et al.* 2014). The uncertainty of the method, expressed as one standard deviation when measuring the same sample 10 times, was 0.2‰ for $\delta^{13}C$ and $\delta^{15}N$, 0.3‰ for $\delta^{34}S$, 2‰ for $\delta^{2}H$ and 0.3‰ for $\delta^{18}O$.

Statistical analyses

We tested the significance of the differences in isotope values between different tissues (claws vs. feathers) using Wilcoxon tests for paired data, and between colonies (five levels) and habitats (three levels) by Kruskal-Wallis tests in which the isotope ratios were considered as dependent variables. We performed hierarchical cluster analysis to arrange individuals into groups based on similarities in stable isotope ratios. We used this method because it does

not require the *a priori* definition of the number of clusters in which individual samples (i.e., feathers or claws) must be arranged. As grouping variables, we considered together the isotope ratio values of the five elements (C, N, S, H, O). We repeated the analysis separately for feather and claw samples.

We than investigated the relationships between isotopic signatures of C, N, S, H and O, by processing linear regression models between each element.

We fitted observed data of δ^2H from chicks feathers on the regression line proposed by Bowen *et al.* 2005 built with the predicted δ^2H of mean annual precipitation water landscape (isoscape) model (Bowen 2010) versus observed δ^2H feather values of local European birds (Hobson *et al.* 2004). As predicted mean value for the δ^2H of precipitation water for southern Sicily we use the value of -30 ‰ (Bowen 2016). We used a single t-test to compare expected value following the equation with the mean real ones observed in our samples. All analyses were carried out in SPSS 13.0 (SPSS inc.) and the significance level was set at $p = 0.05$.

RESULTS

The descriptive statistics of isotope ratios for the five elements and the two tissues (feathers and claws) are reported in Tab. 1. The values of $\delta^{13}C$ were extremely homogeneous across samples and showed standard deviation values below 0.4, which were considerably lower than the much wider range of values for the other four elements (Tab. 1).

The mean isotope ratios of all five elements significantly differed between feathers and claws, as shown by the paired Wilcoxon tests on the six chicks for which double sampling was available (Tab. 2). In particular, values of $\delta^{15}N$, δ^2H and $\delta^{18}O$ were significantly higher in feathers than in claws, whereas the opposite was true for $\delta^{13}C$ and $\delta^{34}S$ (Tab. 2). The hierarchical cluster analysis of feathers and claws separated colony 5, which was surrounded by artichoke fields, from the other colonies (Fig. 1). All chicks from the same nest clustered together in both trees, with one exception (Co2-Ne1-Ch2) in the feather tree (Fig. 1).

Kruskal-Wallis tests showed that isotope ratio differed among colonies for all the elements and for both tissues in all cases ($p < 0.043$ in all cases) with the exception of δ^2H measured in feathers that were analogous across colonies ($p = 0.064$). Isotopic ratios also significantly differed among habitat types for both tissues (Kruskal-Wallis tests, $p < 0.039$ in all cases), with the only exception of $\delta^{34}S$ and δH tested in feathers, which was not statistically different among habitats ($p = 0.197$ and 0.097 respectively).

The relationship between $\delta^{34}S$ and $\delta^{15}N$ points out a remarkable grouping of colonies, coherently to the surrounding habitat and showing negative $\delta^{34}S$ for Monteleone and Torre Vecchia colonies and positive for Settefarine, Giaurone and San Gregorio colonies, while more enriched $\delta^{15}N$ is observed in Giaurone and San Gregorio colonies (Fig. 2).

We found a strong positive relationship between δ^2H and $\delta^{18}O$ ratios ($r^2 = 0.64$ for feathers, $r^2 = 0.76$ for claws). The δ^2H ratio measured in the feathers (mean ± s.e.= -52.7 ± 1.6, N = 13) is comparable to those expected for the generic isoscapes of insectivorous birds (-51.9; Bowen *et al.* 2005, single t-test: p = 0.631).

DISCUSSION

Despite the small sample size, our findings suggest the low consistency of stable isotope ratios between different tissues grown at the same time, and consistent differences in

Table 1. Descriptive statistics of isotope ratios ($\delta^{13}C$, $\delta^{15}N$, $\delta^{34}S$, δ^2H, $\delta^{18}O$) in feathers and claws of Lesser Kestrel chicks. Isotope data are expressed in delta notation (δ).

	$\delta^{13}C$	$\delta^{15}N$	$\delta^{34}S$	δ^2H	$\delta^{18}O$
Feathers (N = 13)					
Min	-23.96	7.16	-4.72	-62.3	15.84
Max	-23.05	12.28	1.8	-40.3	19.09
Mean (S.E.)	-23.55 (0.08)	9.20 (0.45)	-0.93 (0.68)	-52.69 (1.61)	18.03 (0.29)
SD	0.3	1.62	2.44	5.8	1.06
Claws (N = 10)					
Min	-23.95	6.83	-3.55	-96.1	13.92
Max	-22.81	10.43	1.38	-58.32	18.49
Mean (S.E.)	-23.55 (0.13)	8.32 (0.34)	-0.94 (0.67)	-77.60 (4.58)	15.95 (0.53)
SD	0.41	1.07	2.1	14.48	1.69

Table 2. Intra-individual variation in isotope ratios between feathers and claws in six Lesser Kestrel chicks. Reported values of Z and p derive from a Wilcoxon paired test.

	$\delta^{13}C$	$\delta^{15}N$	$\delta^{34}S$	$\delta^{2}H$	$\delta^{18}O$
Mean Feathers (S.E.)		9.35 (0.61)	-2.78 (0.82)		18.24 (0.48)
Mean Claws (S.E.)		7.95 (0.52)	-2.27 (0.66)		16.97 (0.57)
Z	-2.2	-2.2	-1.99	-2.2	-2.2
p	0.028	0.028	0.046	0.028	0.028

colonies from the same population but surrounded by different habitats. The fact that a non-random pattern emerged from such a low sample size is promising for further analyses towards the issues addressed by our tests. Eventually, we interpreted our findings in order to give specific indications on the use of the different elements in future studies on Lesser Kestrel based on the biogeochemical approach.

Different isotopic ratios across tissues (claws and feathers)

The lack of consistency between the isotope ratios for all elements for claws and feathers suggests that isotope signals obtained from the two tissues are not directly comparable. Structural dissimilarities between claws and feathers are unlikely to be the cause of this difference, because both tissues contain extremely similar β-keratin molecules (Bragulla & Homberger 2009). Different tissues are synthesised at different rates and possibly from different dietary components, which might generate a discrepancy in isotope ratios, despite the identical microstructure of the keratins (Inger & Bearhop 2008). A basal difference in $\delta^{2}H$ values between claws and feathers grown at the same time was found in juvenile Blackbirds (Evans *et al.* 2012). Using these data, Evans *et al.* (2012) applied a linear regression equation to rescale the $\delta^{2}H$ values of claws according to basal differences in values of feathers, to make the two values directly comparable. We suggest that analogous regression could be performed to rescale the Lesser Kestrel

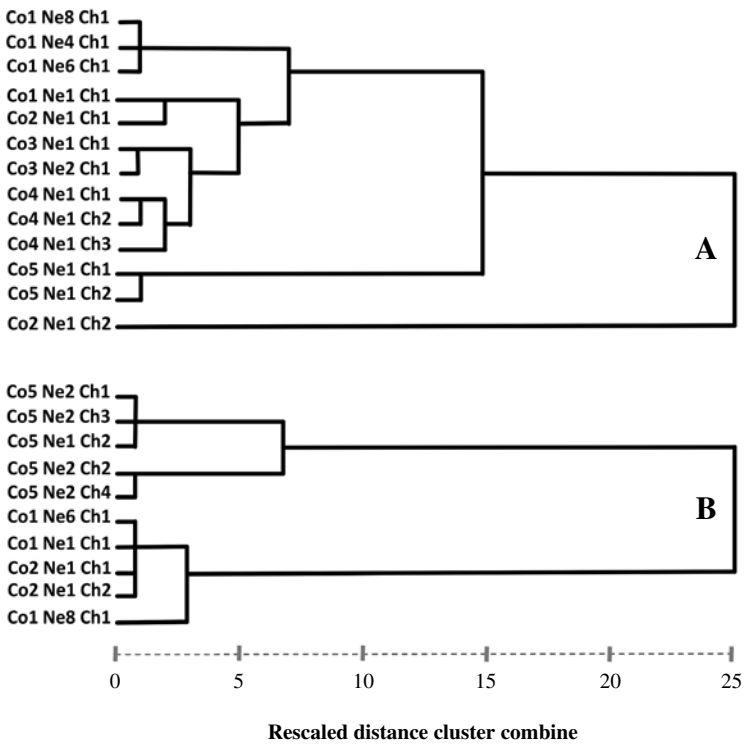

Figure 1. Trees resulting from hierarchical cluster analysis of feathers (**A**) and claws (**B**), using the values of five isotopic ratios ($\delta^{13}C$, $\delta^{15}N$, $\delta^{34}S$, $\delta^{2}H$, $\delta^{18}O$). **Co** = colony, **Ne** = nest, **Ch** = chick.

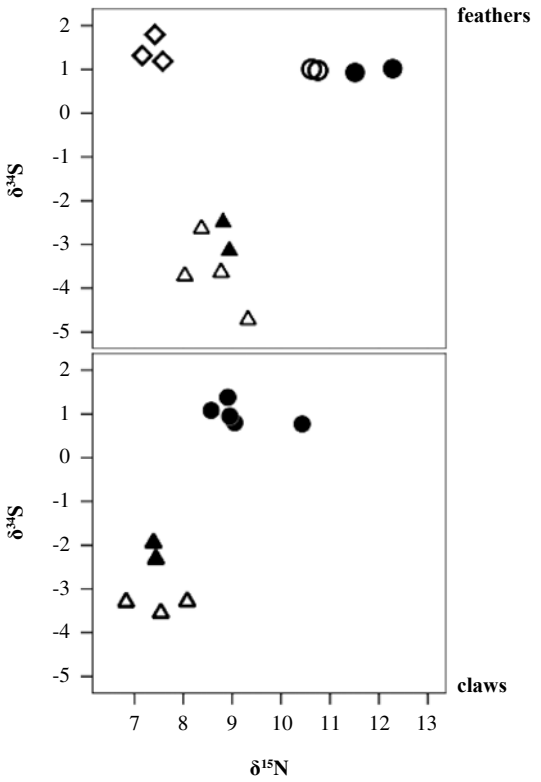

Figure 2. Discrimination among habitats based on $\delta^{15}N$ and $\delta^{34}S$ isotopic ratios in feathers and claws. Different symbols represent each habitat, while empty and full icons separate colonies (see legend).

claw values to make them comparable with those of feathers for all the five studied elements. However, we suggest the possibility to test for claws isotopes only in dead birds as we did, and we advise against the sampling of claws in living raptors, given the plausible great negative impact that this may have on the efficiency of claws in catching preys.

Different isotopic ratios among colonies and among nests of a same colony

Hierarchical cluster analysis confirmed that chicks of the same nest tend to cluster together (see Resano-Mayor *et al.* 2014), similar to nests of the same colony. Less obviously, colonies segregated into two main clusters, depending on the main habitat surrounding the colony, and results based on claws and feathers are analogous. These findings indicate that individuals that breed in the same colony have basically the same foraging habitat. Social raptors such as the Lesser Kestrel usually feed in flocks and use the colony as an information centre (Serrano *et al.* 2004, Calabuig *et al.* 2008). Our findings are consistent with pellet analysis of Lesser Kestrel from the Gela Plain, in which diet composition was significantly different between colonies surrounded by different habitats (Di Maggio *et al.* 2016). Nonetheless, two nests from the same colony significantly differed in isotope ratio values (especially $\delta^{15}N$), therefore, some anomalies from the basic pattern above might exist, showing that individual diet specialisation can occur, even within a colony (Bolnick *et al.* 2003, Bearhop *et al.* 2006), with the adults breeding nearby using different feeding areas or different prey within the same area.

Habitat management highly influences the breeding performance and colony occupation of Lesser Kestrels (Sarà 2010, Catry *et al.* 2013) and the maintenance of suitable foraging habitats is fundamental for the conservation of this species (Franco & Sutherland 2004). Our findings suggest an overall high consistency in the feeding behaviour within a colony, and that the feeding opportunities in the immediate vicinity of the colonies directly affect food availability for chicks, and suggest that the correct management of this spatially limited buffer might confer great conservation benefits.

Isotopic ratios reflect differences in the habitat surrounding the colonies

We found that variability of the $\delta^{13}C$ among sample was extremely low, so that extreme values only differed by less than 1‰. The relationship between $\delta^{13}C$ and $\delta^{2}H$ suggest that these two isotopes could be candidates to distinguish more arid environment, where evaporation leads to more enriched values for water hydrogen and plants are subject to stress that increase heavy fraction of carbon in their tissues (Männel *et al.* 2007), compared to wet environments. This fact could suggest a different agricultural management, with presence of irrigated cultures, especially for San Gregorio and Torre Vecchia colonies hunting areas, while a more xeric environment for Giaurone, Settefarine and Monteleone colonies.

Little research has been performed in ornithology on the variability and absolute values of $\delta^{34}S$ (Hobson 2011), and studies have used sulphur isotopes mostly as markers for assessing marine-derived nutrients (Lott *et al.* 2003, Hebert & Wassenaar 2005). The relatively higher $\delta^{34}S$ val-

ues detected in samples from colonies surrounded by intensively-grown artichoke fields might relate to the high amount of fertilisers used in this type of cultivation (Lo Giudice *et al.* 2014), which might contain large quantities of marine sulphate with high $\delta^{34}S$ values. The higher values in grassland samples are more difficult to interpret, but might be related to the specific geological characteristics of the soil where these colony members usually hunt. Indeed, the Gela Plain is geologically peculiar, as rocks derived from fluvial deposits (carrying low $\delta^{34}S$ values) as well as marine-derived rocks (carrying high $\delta^{34}S$ values) are both present (International Quaternary Map of Europe, IQuaME2500). It could be argued that the Kestrel hunting area of the grassland colony is located in a zone containing marine-derived rocks. The segregation of the only grassland colony from the others under the isotopic profiles should be considered as a preliminary results, being further sampling of other grassland-located colonies needed prior to take sound conclusions on this point.

The relationship between sulphur and nitrogen isotopes brought out an evident differentiation among colonies in isotopic composition. This suggests a strong relationship with habitats used by different colonies. Two extremely different areas are underlined by sulphur isotopes, and coincidentally the two colonies with negative values are both on the orographic left of the Gela plain, while the other three on the right side.

Nitrogen is typically the most informative element in avian dietary analysis (Hobson 2011). Indeed, findings relating to $\delta^{15}N$ are widely used in ecological studies to determine the trophic levels of animals (Kurle & Worthy 2002, Post 2002). Differences in $\delta^{15}N$ among colonies are thus likely to represent different prey collected by Kestrels, depending on their foraging habitat. The evaluation of these findings should also consider that the anthropogenic input of N (i.e., fertilizer) can affect the isotope composition of the local food web (Naddelhoffer & Fry 1988, 1994). In fact, we found notable $\delta^{15}N$ enrichment in chicks raised in colonies surrounded by intensively-grown artichoke fields, where a huge amount of organic fertilizers are used (Lo Giudice *et al.* 2014).

Specific use of different elements (C,O,H) in future biogeochemical studies on Lesser Kestrel

In general, $\delta^{13}C$ ratio in feathers and claws shows values typical of a C3 plant association, as it could be expected in a European grown bird (Still & Powell 2010). For a migratory species such as the Lesser Kestrel, the presence of a marker such as $\delta^{13}C$, which characterises an entire breeding area, can be used to discern tissues grown in breeding quarters (Europe) from those grown in wintering areas (Africa). Western European populations of Lesser Kestrel overwinter in the Sahel region, where they select xeric and subtropical grasslands (Rodriguez *et al.* 2009, Limiñana *et al.* 2012), which are mainly dominated by C_4 vegetation (Still & Powell 2010). This suggests that feathers that moult in wintering quarters should contain C_4 signals, which differ from those of tissues generated in the European breeding quarters dominated by C_3 plants (Still & Powell 2010). Consequently, the analysis of $\delta^{13}C$ values in tissues generated during the wintering periods offers the possibility to distinguish birds that have wintered north of the Sahara from those that normally migrate to the Sahel, potentially allowing the study of partial migration in Lesser Kestrel populations (Negro *et al.* 1991, Tella & Forero 2000). Indeed, to this method to be useful, a deep knowledge of the moult pattern of Lesser Kestrel is needed, while this is actually lacking in literature. Recent evidences that associated stable-isotopes tests to GPS tracking of individuals showed that sampling wing feathers may bring to misleading results, so that European C3 signal may be found in feathers of birds that overwintered in Africa (Gilbert *et al.* 2015). Overall, our findings support that the use of $\delta^{13}C$ in Lesser Kestrel tissues represent those expected for the C3 European vegetation, suggesting this element as a good geographical indicator.

The deuterium values ($\delta^{2}H$) found in our samples are fully compatible with the Western Europe $\delta^{2}H$ isoscape of Mallard (*Anas platyrhynchos*) (Van Dijk *et al.* 2014) and Blackcap (*Sylvia atricapilla*) feathers (de la Hera *et al.* 2012, Morganti *et al.* 2015). At the time of plumage moult, two primary consumers, i.e., the Mallard and the Blackcap, share the same isotope pattern with respect to $\delta^{2}H$ of an exclusive predator, i.e., the Lesser Kestrel, which leads to the conclusion that the $\delta^{2}H$ ratio changes little across trophic levels. The range of $\delta^{2}H$ values from our samples appears to be extremely large if we consider that all the individuals come from the same locality and that $\delta^{2}H$ is normally used to establish geographical origin, but variability of up to 50 or 60‰ is normally observed for $\delta^{2}H$ values in samples from a single locality (Hobson *et al.* 2012). On the other hand, $\delta^{2}H$ seems to nicely fit on the isoscapes traced by Bowen *et al.* (2005) for $\delta^{2}H$ in feathers of European land birds. This fact suggests that $\delta^{2}H$ of feathers could be useful for studies that aim to detect origin of wintering Lesser kestrel in Africa that could rebuild an assignment (Bowen *et al.* 2014) by sampling on juvenile feathers in wintering quartiers. Moreover, we observed a strong relationship between $\delta^{2}H$ and $\delta^{18}O$ and a weaker relationship between $\delta^{18}O$ and $\delta^{13}C$. This fact suggests that other factors may contribute to final signal in $\delta^{18}O$, as differential respiration rate at the individual level (Hobson & Koehler 2015).

Concluding remarks

In this study, we show that an isotope approach to Lesser Kestrel ecology is highly promising. Our findings suggest that future studies should carefully select the tissues that will be the object of study or consider that the immediate habitat surrounding the breeding sites might strongly determine the isotope composition of the sampled tissues. This knowledge will help to provide a correct application of methodology in future research. Moreover, our findings explicitly propose that $\delta^{13}C$ should be used to study partially migratory patterns in the Lesser Kestrel, which are of growing interest, given the expected increase in the proportion of the resident individuals in southern European populations that face climate change (Negro *et al.* 1991, Morganti 2015). The $\delta^{15}N$ and $\delta^{34}S$ isotopes are instead suggested for current use to estimate differences in the diet, which might arise from the use of different habitats or from different feeding preferences within the same habitat. In the future, we call for the large sampling of feathers of living birds and potential prey across the breeding and overwintering habitats in the range of the Lesser Kestrel, with the aim to trace a reliable isoscape for this species. This would represent an important tool to for the ecological study of this delicate raptor and potentially enhance its long-term conservation.

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