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Sexual and parent-offspring dietary segregation in a colonial raptor as revealed by stable isotopes

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Summary

Diet composition and foraging behaviour may show considerable variation among population groups (such as sex- and age-classes), with potentially important consequences for population dynamics. Thus, failure to account for within-species differences in trophic ecology can bias our understanding of different aspects of population ecology and limit the implementation of effective management and conservation strategies. Although countless studies have investigated the diet of birds, comparatively few have tried to describe intraspecific sources of dietary variation. Here, we used stable isotope analysis (SIA) to investigate sex- and age-related dietary segregation in the lesser kestrel (*Falco naumanni*) breeding in South Iberia and to discuss potential mechanisms involved in such segregation. Females had a narrower isotopic niche width and significantly more depleted $\delta^{13}C$ signatures than males during the courtship period, likely due to a higher consumption of energetically rich mole crickets. Our results suggest that sex-specific differences in the diet of lesser kestrels do not result from intra-specific competition and are unlikely to be explained by sexual size dimorphism alone. Instead, the main driving force of observed sexual segregation appears to be the different energetic requirements of males and females before laying, when females need a higher allocation of resources to egg production. $\delta^{15}N$ isotopic signatures differed significantly between adults and chicks and niche overlap between these age classes was low. Stable isotopic mixing models (SIAR) showed that, compared to adults, the diet of chicks was less diverse and mainly dominated by grasshoppers. Different resource allocation between chicks and adults might also result

from different energy requirements, as rapidly growing chicks require more energy than adults, ultimately leading to a parent-offspring dietary segregation. Finally, overall agreement between pellet analysis and SIA methods highlight the potential of SIA for assessing intra-specific variation in dietary regimes which is often unfeasible through conventional approaches of diet assessment.

Introduction

Among bird populations, dietary segregation between sexes or age groups has often been reported (Forero *et al*., 2002; Alonso *et al*., 2012; Catry, Phillips & Croxall, 2005; Rey *et al*., 2012; Beaulieu & Sockman 2014). Trophic sexual segregation is more spread among sex-dimorphic species and often linked to niche specialization arising from different morphology or the different roles played by males and females during reproduction (Forero *et al*., 2002; Phillips *et al*., 2004). Alternatively, sexual segregation might be driven by social dominance or competitive exclusion, where larger or more aggressive individuals exclude inferior competitors from high quality foraging areas (González-Solís, Croxall & Wood, 2000). Regardless of the main drivers involved in its genesis, dietary segregation between sexes is often a proficient mode of reducing intra-specific competition (Catry *et al*., 2005), especially when the potential for competitive interactions is maximum, such as for central-place foragers during reproduction, an energetic demanding period. In species with reversed sexual size dimorphism, previous studies suggested that larger females might be able to capture larger prey, while smaller body-sized males gain on agility, being well adapted for the pursuit and hunting of smaller prey (Newton, 1979), hence reducing competition for food. Diet segregation between sexes can also potentially arise in a particular period of the breeding season, when male and females have different energetic needs owing to

different roles in the breeding cycle (Weimerskirch *et al.*, 2009; Ludynia *et al.*, 2013). In many species, females are fed by males during periods of high-energy expenditure (Galván & Sanz, 2011), such as courtship or incubation periods. Higher nutritional requirements of females could lead to a biased prey choice by males, by delivering the rich-energy prey to their mates and consuming the smaller and less nutritional items.

Dietary age segregation, and in particular parent-offspring segregation, has been described to reflect the different nutritional needs of adults and chicks, with the later requiring higher-quality prey for a fast developing and improved survival probability (Alonso *et al.*, 2012; Rey *et al.*, 2012; Beaulieu & Sockman 2014). On the other hand, central place foragers are often single-prey loaders, and should thus maximize their rate of energy delivered per provisioning trip (Stephens & Krebs, 1986). As a consequence, prey delivered to chicks are frequently larger and more nutritive than the average consumed by parents (Wilson, Daunt & Wanless, 2004) thus reducing the flight and transport costs associated with provisioning and at the same time fulfilling the energy demands of growing chicks.

Diet analyses are central to the study of foraging ecology but intra-specific dietary segregation studies are largely biased towards a few groups, namely seabirds and shorebirds. Among raptors, sex and age have only occasionally been incorporated into general studies of diet, despite clear evidence of inter-sexual and age-related habitat segregation and differences in foraging behaviour (Ardia & Bildstein, 1997; Buij *et al.*, 2012). Whist most studies with raptors fail to focus on intra-population variation, this information is relevant to understand their life-histories and population regulation, with clear implications for management and conservation (Newton, 1979; Marti, Bechard & Jaksic, 2007; Buij *et al.*, 2012). Failure to account for within-species differences in diet are due to limitations of the sampling techniques used, namely analysis of faecal

droppings and regurgitated pellets. In addition of being potentially biased towards large and low digestible prey (Marti *et al*., 2007), in most cases these approaches prevent drawing the link between dietary remains and particular individuals, hindering the study of diet segregation among intra-population groups. More recently, a complementary approach, based on the use of stable isotopes analysis (SIA), in particular of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$), allowed a relevant advance in studies of avian trophic ecology (e.g. Hobson & Clark 1992; Bearhop *et al*., 2004), both overtaking previous methodological bias and allowing identification of intra-population variation in diet. Isotopic ratios in bird tissues reflect its diet during the period of tissue synthesis (Hobson & Wassenaar, 2008). Blood, being a metabolic active tissue, typically yields an isotopic record of some days to few weeks prior to collection date (Hobson & Wassenaar, 2008), which might be ideal for studies of trophic ecology during the breeding season, with different sampling events allowing diet characterization throughout the whole season. The lesser kestrel is a small colonial migratory raptor with reversed sexual size dimorphism (Newton, 1979). As in other raptor species, during the mate-feeding period males may select larger and more energetic prey to feed their mates so they can reach the energy requirements needed for egg production and laying (Newton, 1979; Donázar *et al*., 1992). After laying, females and males share incubation and chick feeding duties until the young fledges. Whilst lesser kestrel diet has been studied in several countries and seasons (e.g. Rodríguez *et al*., 2010; Lepley *et al*., 2000), no studies have evaluated age and sex-related differences as sources of variation in the diet of this species. Here, we used SIA to investigate sex and age-specific dietary segregation in the lesser kestrel (*Falco naumanni*) during the breeding season in south Iberia. We hypothesized that size dimorphism and different roles played by male and female lesser kestrels during the breeding cycle may lead to differences in energetic

requirements and likely promote inter-sexual differentiation in diet composition. We further hypothesized that as central place foragers, lesser kestrels are selective in relation to prey delivered to their offspring, which can lead to dietary segregation between adults and nestlings. Finally, we compared SIA and conventional pellet analyses to assess the overall agreement between these two techniques in describing temporal shifts in birds' diet.

Methods

Study area and data collection

This study was conducted in the Castro Verde Special Protection Area (SPA), Southern Portugal. Here, lesser kestrel colonies are found in ruins of abandoned farmhouses or artificial nesting structures scattered in a cereal steppe landscape (Catry *et al*., 2009). Breeding birds arrive from their African wintering areas in early February and typically lay in April and May. Incubation takes 28 days and after hatching both parents feed the chicks for about 35-37 days (Bustamante & Negro, 1994).

Blood sampling for SIA analysis was carried out during the breeding seasons of 2013 and 2014 (Table 1). Adults (n = 8 and 130 in 2013 and 2014, respectively) and chicks (n = 10 and 22 in 2013 and 2014, respectively) were caught on their nests and ~150 µl of blood was collected from the brachial vein. Adults were sampled during courtship, incubation and chick rearing periods (in 2013 adults were caught only during the chick rearing period; Table 1). Based on the allometric relationship between elemental turnover and body size described by Hobson & Wassenaar (2008), we assumed that whole blood of lesser kestrels has a half-life of approximately two weeks. Thus, blood samples were presumed to give information on diet composition of lesser kestrels for approximately 15 days before the sampling event. This was taken into

account when establishing the periods for dietary analyses (courtship, incubation and chick rearing). All sampled birds were ringed and weighted at the time of capture. Lesser kestrel nests where blood sampling took place were monitored on a weekly basis throughout the breeding season to assess laying date and clutch size. In 2014, the maximum length (L) and breadth (B) of eggs were measured to the nearest 0.01 mm using a digital calliper and egg volume was afterwards estimated as Vol (mm^3) = K × L (mm) × B^2 (mm) where K = 0.51 (Catry, Franco & Sutherland, 2012).

To characterize isotopically the main prey of lesser kestrels, we collected samples from the most widely consumed prey found in the nests in 2013 and 2014 (Table 2). Lesser kestrels feed predominantly on invertebrates, but also on small mammals and reptiles (Lepley *et al*., 2000; Rodríguez *et al*., 2010). Previous work in our study area (Teodósio, 2000; Catry *et al*., 2012) and pellet analysis (this study) showed that mole crickets (Gryllotalpa sp.) and short or long-horned grasshoppers (Acrididae and Tettigonidae, hereafter grasshoppers) but also small mammals (rodents and shrews, Muridae and Soricidae families, respectively), Western three-toed skinks (*Chalcides striatus*), scolopendras (Scolopendridae family) and beetles (Coleoptera) are the main prey consumed during the breeding season. Thus, these items were selected for SIA analysis and for lesser kestrel diet reconstructions using Bayesian mixing models (SIAR, see below). Blood and prey samples were frozen immediately after collection.

To assess the level of agreement between SIA and pellet analysis, we compared the temporal variation in blood isotopic signatures and in the occurrence of mole crickets and grasshoppers in pellets. Mole crickets and grasshoppers were used as they are primary prey of lesser kestrels (high frequency and biomass) and their abundance is easy to quantify in pellets. Lesser kestrel pellets were collected every two-weeks in three colonies where blood sampling took place, from 7 March to 4 July in 2014, thus

spanning the entire breeding season for most pairs. In each visit and colony, we collected 20 pellets from a pre-selected area comprising 50–100% of all occupied nests (to ensure a representative sample from each colony). Overall, we analysed 420 pellets and the mean number of mole crickets and grasshoppers per pellet in each colony was assessed by counting the number of mandibles or other non-digestible pieces with the help of a magnifying glass (Rodríguez *et al*., 2010; Catry *et al*., 2012).

Stable isotope analysis

All samples (lesser kestrel blood and prey muscle) were dried in an oven at 60ºC for 24-48h and afterwards ground into a homogeneous powder using mortar and pestle. Lipid extraction was performed in all prey samples, which were immersed in a 2:1 chloroform/methanol solution with a solvent volume 3–5 times larger than sample volume (Logan *et al*., 2008). Samples were then mixed for 30s, left undisturbed for approximately 30 min, and centrifuged for 10 min at 3400 rpm, and the supernatant containing solvent and lipids was removed. This process was repeated at least three times until the supernatant was clear and colourless following centrifugation. Samples were re-dried at 60ºC for 24h to remove any remaining solvent. Between 0.9 mg and 1.2 mg of each sample was stored in tin cups for stable carbon and nitrogen isotope assays. Isotopic ratios were determined by continuous-flow isotope-ratio mass spectrometry (CF-IRMS). Results are presented conventionally as δ values in parts per thousand (‰) relative to the Vienna Pee Dee Belemnite (VPDB) for $\delta^{13}C$, and atmospheric nitrogen (N2) for $\delta^{15}N$ (Tables 1 and 2). Measurement precisions were 0.13 to 0.32‰ for $\delta^{13}C$ and 0.07 to 0.26‰ for $\delta^{15}N$ (SD).

Assessing diet composition

To estimate the relative contribution of each prey to the diet of lesser kestrels (by sex, age and breeding state) we run Bayesian mixing models with the SIAR package for R (Parnell *et al*., 2008). Sampled prey were included in the diet reconstructions for each breeding state (courtship, incubation and chick rearing) if they were present in pellets; thus, grasshoppers and mole crickets were excluded from SIAR models for the courtship and chick rearing periods, respectively; all other prey were included. Trophic discrimination factors used to run mixing models are known to vary with consumer, tissue and diet quality (e.g. Hobson & Clark, 1992). Given the absence of previous studies experimentally determining discrimination factors in lesser kestrels (or in bird species with similar dietary composition) we estimated the mean (±SD) value of trophic discrimination factors for blood samples of several bird species reported in published studies following a wide bibliographic review (see Supporting Information, Appendix 1). Accordingly, we used discrimination factors of 2.37 ± 0.62 for $\delta^{15}N$ (n=13 species*diet treatments) and 0.59 ± 1.09 for $\delta^{13}C$ (n=16 species*diet treatments). Given that SIAR produces a probability distribution of solutions based on uncertainty in SD values, the relatively high SD in our case provides a conservative approach to study diet composition of lesser kestrels.

To measure and compare isotopic niche of lesser kestrels among distinct groups (adults versus chicks in 2013 and 2014, and males versus females during courtship and during incubation in 2014) we estimated areas of standard ellipses, which contain ca. 40% of all data and are less sensitive to extreme values and low sample size than total area (Jackson *et al*., 2011). We calculated (1) Bayesian standard ellipse areas (SEA_B), which can be compared in a quantitative manner (Jackson *et al*., 2011), and (2) small sample size-corrected standard ellipse areas (SEA_C). In addition, we assessed overlap in SEA_C between lesser kestrels groups. For each group (*i*) in one pair (*i,j*), a value of

overlap ($Ov_{[i]}$) was calculated as the ratio between the area of overlap between the two SEA_C ($A_{[i, j]}$) and its own SEA_C ($A_{[i]}$),, expressed as a proportion ($Ov_{[i]} = A_{[i,j]} / A_{[i]}$). Differences between males and females during the chick rearing period could not be explored due to the small sample size of males (n = 3).

Statistical analyses

We used two-way ANOVAS (followed by post-hoc Tukey tests) to test for sex and breeding state (courtship, incubation and chick rearing periods) differences on carbon and nitrogen isotopic signatures of adult lesser kestrels. Moreover, differences in isotopic signatures between mates sampled simultaneously during courtship were assessed using paired t-tests. We also used t-tests to assess age-class (chicks versus adults) differences in isotopic signatures during the chick rearing period.

Generalized linear models (GLMs) were used to assess the relationship between $\delta^{13}C$ values of females during courtship and body weight and between $\delta^{13}C$ values of females during laying and mean egg volume per clutch. Laying date was firstly included as a second predictor, because we expected egg volume to decrease with laying date, but removed afterwards given its non-significance.

Temporal variation in blood isotopic signatures of lesser kestrels and in the occurrence of mole crickets and grasshoppers in pellets was assessed using generalized additive models (GAMs). From the beginning of April, $\delta^{13}C$ and $\delta^{15}N$ signatures of adult birds sampled and the number of mole crickets and grasshoppers per pellet along the breeding season were modelled using Gaussian distribution and identity-link functions. All models were fitted using the ‘‘mgcv’’ package and the “gamm” function (Wood, 2006). A basis dimension of k = 4 and a gamma value of 1.4 was set for the non-linear term to allow some complexity in the function while providing a realistic

prediction of temperature effects and avoiding over-fitting of the data (Wood, 2006). Residuals of final models were visually inspected to ensure model assumptions were met.

All analyses were conducted in the R statistic environment (R Development Core Team, 2013).

Results

We found significant differences in $\delta^{13}C$ and $\delta^{15}N$ signatures of adult lesser kestrels among breeding stages but not between sexes (two-way ANOVA: breeding stage $F_{2, 124} = 8.85$, $P < 0.001$; sex $F_{1,124} = 3.10$, $P = 0.08$ and breeding stage $F_{2,124} = 8.56$, $P < 0.001$; sex $F_{1,124} = 0.60$, $P = 0.44$, for $\delta^{13}C$ and $\delta^{15}N$, respectively), although the interaction effects between both variables were significant ($F_{1,124} = 3.53$, $P < 0.05$ and $F_{1,122} = 3.40$, $P < 0.05$, for $\delta^{13}C$ and $\delta^{15}N$, respectively). Indeed, females showed significantly more depleted $\delta^{13}C$ values than males during the courtship period (post-hoc tests $P < 0.05$) while sex-related differences were not apparent during incubation (post-hoc tests $P = 0.94$). Comparisons between mates of known breeding pairs captured in the same day during courtship also showed significant lower $\delta^{13}C$ values amongst females ($\delta^{13}C$: $t = -2.71$, $P < 0.05$, $\delta^{15}N$: $t = 0.34$, $P = 0.74$; $n = 10$). Overall, $\delta^{13}C$ values were significantly lower during courtship in comparison with the other periods (post-hoc tests $P < 0.01$, Table 1) whilst $\delta^{15}N$ values were significantly lower during the chick rearing period (post-hoc tests $P < 0.01$, Table 1).

Results from SIAR mixing models revealed that females consumed a significantly higher proportion of mole crickets than males during the courtship period (modal values: 55 and 35% for females and males, respectively; Fig. 1). During this

period, males showed a broader isotope niche width (as estimated by SEA_B), almost twofold the niche width of females (Table 3).

There is a negative significant association between individual body weight and $\delta^{13}C$ values in female lesser kestrels during courtship ($R^2 = 0.22$, $F_{1,53} = 14.85$, $P < 0.001$; Fig. 2), suggesting that females with diets rich in mole crickets (highly depleted in $\delta^{13}C$, Table 2) have a better body condition. We found that females with increased body weight (and lower $\delta^{13}C$ values) have increased mean egg volume per clutch ($R^2 = 0.34$, $F_{1,13} = 6.55$, $P < 0.05$; Fig. 2). During incubation, the consumption of mole crickets decreased notably (modal values: 19 and 23% for females and males, respectively Fig. 1) and grasshoppers became the most important prey for both male and female lesser kestrels (modal values: 31 and 30% for females and males, respectively Fig. 1). Isotopic niche width was similar between sexes, with substantial overlap (Table 3). The importance of grasshoppers in the diet of females peaked during chick rearing (modal values: 52%, Fig. 1). Comparison with males is not shown due to small sample size (see methods).

Comparison of isotopic signatures between adults feeding chicks and chicks depicted significant differences in $\delta^{15}N$ ($t = 2.43$, $P < 0.05$ and $t = 3.78$, $P < 0.01$ for 2013 and 2014, respectively) but not in $\delta^{13}C$ values ($t = 0.79$, $P = 0.44$ and $t = -0.34$, $P = 0.74$ for 2013 and 2014, respectively; Fig. 3) in two consecutive breeding seasons. SIAR mixing models show that observed higher $\delta^{15}N$ values of chicks reflect a higher proportion of grasshoppers in their diet (modal values: 23 and 52% in 2013, 52 and 61% in 2014, for adults and chicks, respectively; Fig 4 and Table 2). Dietary segregation among adults and chicks during chick rearing was particularly evident in 2013 (Fig. 4). Nestlings displayed narrower isotopic niche width when compared to adults (Table 3).

Mean niche overlap between age classes was low (no overlap in 2013), suggesting an age-specific partitioning in resource use (Table 3).

Comparison of SIA and pellet analysis

Blood isotopic signatures of adult lesser kestrels changed significantly across the breeding season, showing a shift towards higher $\delta^{13}C$ and lower $\delta^{15}N$ values (GAM: edf = 1.99, F = 13.22, P < 0.001 and edf = 1.31, F = 9.36, P < 0.001 for $\delta^{13}C$ and $\delta^{15}N$, respectively; Fig. 5). This is in line with the recorded shift in the dominance of mole crickets ($\delta^{13}C$ depleted) during the early season, especially during courtship, towards a grasshopper-dominated diet ($\delta^{15}N$ depleted) during incubation and chick rearing (Fig. 1), as revealed by SIAR mixing models. Temporal changes in the dietary regime of lesser kestrels depicted by SIA are highly supported by the results of pellet analysis (Fig. 5b). Mole crickets consumption (mean number of mole crickets per pellet) decreased significantly from the beginning to the end of the breeding season (GAM: edf = 2.07, F = 28.88, P < 0.001), contrasting with the significant increase in the consumption of grasshoppers (GAM: edf = 1.18, F = 22.12, P < 0.001; Fig. 5).

Discussion

Among raptors, intraspecific patterns of food allocation between males and females or between parents and their offspring remain broadly unknown. This lack of information is partly due to the difficulty in assigning diet composition to age or sex groups using traditional methods such as pellet analysis. Through a SIA approach, we found compelling evidence of trophic sexual and age segregation in the lesser kestrel, a small migratory falcon with reversed sexual size dimorphism.

Diet segregation between sexes

During courtship, female lesser kestrels showed significantly more depleted $\delta^{13}C$ signatures than males, likely due to a higher consumption of mole crickets, while males showed a wider isotopic niche. Dietary sexual segregation seemed to dissipate with the progress of the season; during incubation diet preferences were broadly alike in male and female kestrels, as revealed by similar $\delta^{13}C$ and $\delta^{15}N$ signatures and high overlap in niche width.

Sexual segregation in foraging behaviour is generally considered to result from social dominance and competitive exclusion or from niche specialization arising from differences in morphology or reproductive role (Catry *et al*., 2005; Rey *et al*., 2012; Ludynia *et al*., 2013). In sexually size-dimorphic species, such as the lesser kestrel (females are slightly larger than males) the larger sex often requires a higher absolute energy intake than the smaller sex to meet metabolic requirements imposed by a larger body mass. Accordingly, if body size differences were a major driver of sex-related dietary differences among lesser kestrels, we would expect differences between the sexes to be maintained across the whole breeding season. However, the lack of dietary sexual segregation outside the courtship period does not provide support to this hypothesis. Niche divergence to reduce intraspecific competition seems also unlikely to explain sexual segregation in lesser kestrels. Intraspecific competition can be avoided by using different prey types and foraging habitats (Rey *et al*., 2012; Ludynia *et al*., 2013). However, in our study area, foraging areas of male and female lesser kestrels are known to overlap broadly during the breeding season (Franco *et al*., 2004). Moreover, females stay much of the time in the colony without hunting before laying, a period in which they are mostly dependent on prey delivered by males (Dónazar *et al*., 1992). The

fact that dietary segregation between sexes was exclusively recorded during the courtship period, strongly suggests it is linked to the mate-feeding behaviour found in this species. The primary function of mate-feeding in the lesser kestrel seems to be the increase of female body mass, possibly to allow the laying of earlier and larger clutches (Dónazar *et al*., 1992). Thus, during courtship, males often select larger and more energetic prey to feed their mates (Lepley *et al*., 2000; Rodríguez *et al*., 2010), keeping the smaller ones for themselves. Mole crickets are large, rich in proteins and lipids (Lepley *et al*., 2000) and one preferred prey of lesser kestrels during courtship, likely playing an important role in the accumulation of energy needed for egg production and laying (Teodósio, 2000; Rodríguez *et al*., 2010; Catry *et al*., 2012). Moreover, previous work found that higher mole cricket consumption was associated with earlier egg-laying dates and larger clutch sizes and egg volume (Catry *et al*., 2012), with likely positive effects on offspring fitness. Hence, sex-specific dietary composition seems to reflect the different energetic requirements during the courtship period and associated sex-specific prey preferences, such as energetically rich mole crickets for females. Our results support this hypothesis, as female body condition during courtship and mean egg volume per clutch were negatively correlated with $\delta^{13}C$ ratios in females' blood, peaking amongst females whose $\delta^{13}C$ values approached those expected from a diet dominated by mole crickets. After egg laying, male and female lesser kestrels share incubation duties and each sex forages for its own nourishment. Similar energetic requirements and prey preferences may thus explain similar diets and a higher dietary overlap at this stage.

Diet segregation by age

Lesser kestrels showed parent-offspring dietary segregation during the chick-rearing period. Compared to adults, chicks showed narrower isotopic niches, with less diverse diets, mainly dominated by grasshoppers. Such segregation was particularly evident in 2013, when there was no isotopic niche overlap between adults and nestlings. Among birds, central-place foragers may optimize their success and their investment in offspring by provisioning themselves with different prey than they feed their chicks, as growing chicks usually demand more energy than adult subsistence (Forero *et al*., 2002; Alonso *et al*., 2012). Also, optimal foraging theory predicts that trade-offs amongst prey value, searching time and prey handling time likely constrain foraging decisions (Stephens & Krebs, 1986). The preference of adult lesser kestrels to feed their offspring a higher proportion of grasshoppers compared to that consumed by themselves might be explained by the relatively large size, low handling time and high energetic value of this prey type (Catry *et al*., 2012; I. Catry, unpublished data). By contrast, when feeding for themselves, and free from returning to their nest after each prey captured, adults can be more opportunistic, consuming a higher diversity of prey, which might also relieve intra-specific competition. Previous studies have also suggested that adult birds may select soft-bodied prey with higher digestibility (such as chitin-free invertebrates) to feed their offspring, keeping the less profitable prey (more chitinized and smaller), such as Coleoptera, for themselves (e.g. Orłowski, Rusiecki & Karg, 2014). Since mole crickets are no longer available during the nestling period (Catry *et al*., 2012), grasshoppers are likely the best prey available to meet the energetic requirements of rapidly growing nestlings.

Comparison of SIA and pellet analysis

Blood ratios of carbon and nitrogen stable isotopes point for a temporal diet shift in lesser kestrels, with mole crickets as a dominant prey early in the breeding season being replaced by grasshoppers later on. These results show an overall agreement with pellet composition, also supporting previous findings (Rodríguez *et al*., 2010; Catry *et al*., 2012). Several methodological approaches, each with specific advantages and disadvantages, are frequently used to study the diet of birds (e.g. Barrett *et al*., 2007). Most raptors regurgitate indigestible prey remains in discrete pellets which are widely used to study their diet (Marti *et al*., 2007). Whilst pellet analysis is a non-invasive technique, simple and which can provide large samples over time, the method can produce biased results given the variability in prey digestibility (Redpath *et al*., 2001; Marti *et al*., 2007). On the other hand, SIA of bird tissues can provide valuable information on its diet composition at the time of tissue synthesis, but prior knowledge on the diet of the target species and isotopic signatures of prey is required (Resano-Mayor *et al*., 2014). Thus, this approach usually requires tissue collection of both predator and prey. Nonetheless, the main advantage of stable isotope analysis through other conventional techniques is the possibility to obtain data from particular individuals, allowing comparing food allocation between males and females or between parents and their offspring. Overall, SIA can be strengthened by other complementary methods (e.g. pellet analysis) to assess dietary composition and seasonal diet shifts of bird species, being thus a crucial tool in the study of avian trophic ecology (Resano-Mayor *et al*., 2014).

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Tables

Table 1. Blood carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) stable isotope signatures (mean ± SD) of adult and nestling lesser kestrels sampled in 2013 and 2014 at Castro Verde, southern Portugal.

	$\delta^{13}C$	$\delta^{15}N$	n
2014			
Courtship			
Females	-25.79 ± 0.52	8.63 ± 0.70	55
Males	-25.37 ± 0.65	8.59 ± 0.98	19
Incubation			
Females	-25.30 ± 0.36	8.31 ± 0.85	18
Males	-25.45 ± 0.52	8.33 ± 0.62	18
Chick rearing			
Females	-25.21 ± 0.47	7.56 ± 1.04	17
Males	-25.18 ± 0.13	8.96 ± 1.39	3
Chicks	-25.37 ± 0.41	7.14 ± 0.78	22
2013			
Chick rearing			
Females	-25.76 ± 0.56	9.78 ± 0.52	8
Chicks	-25.65 ± 0.41	7.83 ± 0.71	10

Table 2. Carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) stable isotope signatures (mean ± SD) of main prey of adult lesser kestrels sampled in 2013 and 2014 at Castro Verde, southern Portugal.

		$\delta^{13}C$	$\delta^{15}N$	n
Small mammals				
Muridae e Soricidae				
	2013	-26.71 ± 0.47	10.13 ± 1.58	4
	2014	-25.42 ± 074	7.27 ± 1.80	9
Reptiles				
Chalcides striatus				
	2013	-25.81 ± 0.18	7.85 ± 1.34	2
	2014	-25.38 ± 0.60	8.34 ± 0.51	5
Arthropoda				
Chilopoda				
Scolopendra sp.				
	2013	-26.59 ± 0.25	9.44 ± 0.33	2
	2014	-25.93 ± 0.92	8.67 ± 1.22	3
Coleoptera				
	2013	-27.78 ± 1.33	7.85 ± 1.13	5
	2014	-26.54 ± 1.07	7.42 ± 1.66	18
Orthoptera				
Gryllotalpidae				
Gryllotalpa sp.				
	2013	-28.45 ± 0.19	4.35 ± 0.83	2
	2014	-26.98 ± 0.90	5.88 ± 0.62	4
Acrididae e Tettigoniidae				
	2013	-25.43 ± 0.57	3.39 ± 1.03	4
	2014	-25.41 ± 0.77	3.40 ± 1.14	18

Table 3. Isotopic niches of lesser kestrels as measured by Bayesian standard ellipse areas (SEA_B) and sample size-corrected standard ellipse areas (SEA_C). Pairwise comparisons of SEA_B are presented between adults versus chicks in 2013 and 2014, and males versus females during courtship and incubation in 2014; values presented are the probabilities that SEA_B of the first group is smaller than the SEA_B in the second group (probabilities higher than 0.80 or lower than 0.20 are presented in bold). Niche overlap, expressed as the proportion of the SEA_C of one group in relation to its pair (see methods), is presented for each group. Sample sizes are given in parenthesis.

		SEA_B	Pairwise comparison between SEA_B	SEA_C	Niche overlap
2014					
Courtship	Females (55)	1.26 [0.94 – 1.59]	**0.990**	1.15	0.69
	Males (19)	2.33 [1.36 – 3.41]		2.11	0.38
Incubation	Females (18)	1.46 [0.86 – 2.16]	0.403	1.03	0.67
	Males (18)	1.35 [0.79 – 1.99]		1.03	0.67
Chick rearing	Adults (20)	1.87 [1.14 – 2.68]	**0.102**	1.44	0.31
	Chicks (22)	1.28 [0.79 – 1.83]		0.94	0.48
2013					
Chick rearing	Adults (8)	2.48 [1.05 – 4.26]	**0.195**	1.35	0
	Chicks (10)	1.64 [0.78 – 2.72]		1.04	0

Figure Legends

Figure 1. Relative contribution of prey in the diet of female and male adult lesser kestrels during the courtship, incubation and chick rearing periods as estimated by SIAR mixing models. Black dots represent the mode and boxes present the 50%, 75% and 95% credible intervals. Contributions for males during the chick-rearing period were not estimated given the small sample size (see methods).

Figure 2. Relationship between body weight (g) and mean egg volume per clutch (mm^3) and stable carbon isotopes ($\delta^{13}C$) in female lesser kestrel sampled during the courtship and laying periods, respectively.

Figure 3. Blood carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) stable isotope signatures (mean ± SD) of adult lesser kestrels feeding chicks and chicks sampled in 2013 and 2014 in Castro Verde.

Figure 4. Relative contribution of prey in the diet of adult lesser kestrels feeding chicks and chicks in 2013 and 2014 as estimated by SIAR mixing models. Black dots represent the mode and boxes present the 50%, 75% and 95% credible intervals.

Figure 5. Predicted values (solid lines) and 95% CI (shaded areas) of (A) blood $\delta^{13}C$ and $\delta^{15}N$ stable isotope signatures of adult lesser kestrels and (B) mole cricket and grasshopper consumption (mean number individuals per pellet) along the breeding season in 2014. Fitted curves were predicted by the inferred GAM coefficients, using the actual range of values for the predictor of interest. Ticks in the x-axis represent the

location of observations (points) along the predictors. Black, grey and white dots show adult kestrels sampled in the courtship, incubation and chick rearing periods, respectively. Sampling date follows the Julian date calendar (1 April = 90).

Figure 1

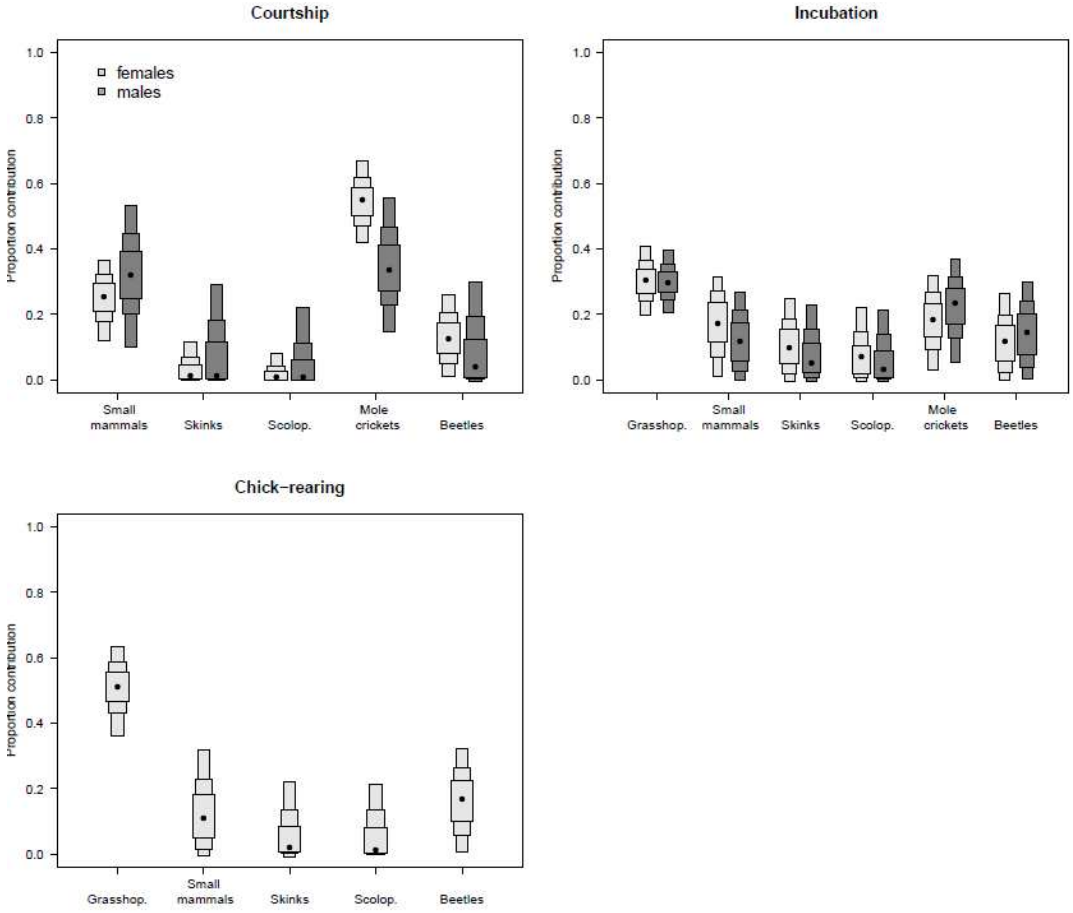

Courtship
females
males
Proportion contribution
1.0
0.8
0.6
0.4
0.2
0.0
Small mammals
Skinks
Scolop.
Mole crickets
Beetles
Incubation
Proportion contribution
Grasshop.
Small mammals
Skinks
Scolop.
Mole crickets
Beetles
Chick−rearing
Proportion contribution
Grasshop.
Small mammals
Skinks
Scolop.
Beetles

Figure 2

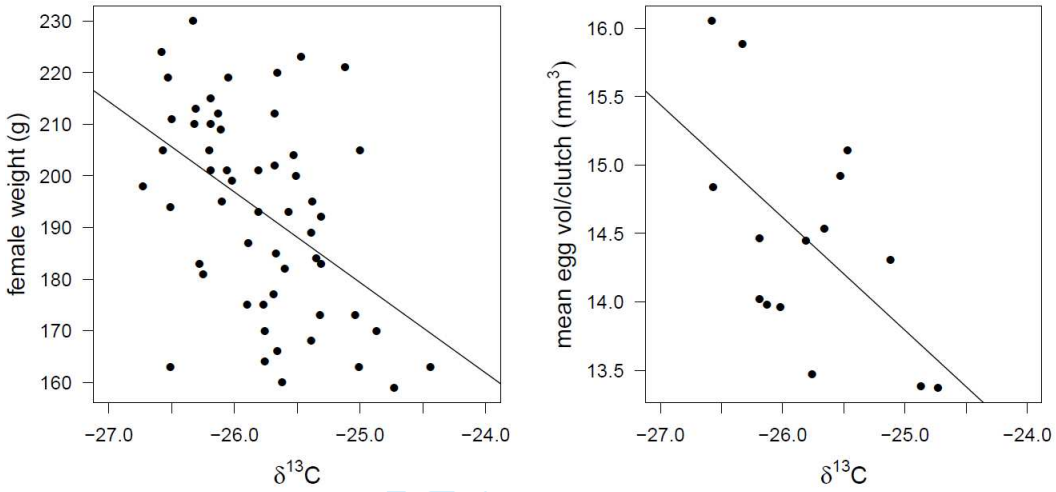


Figure 3

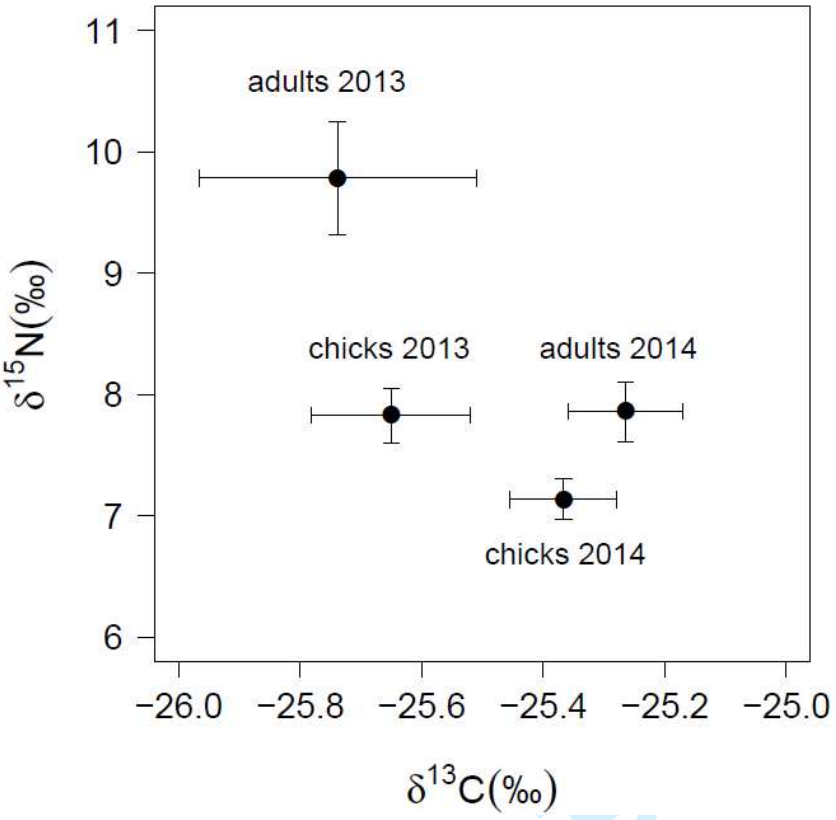
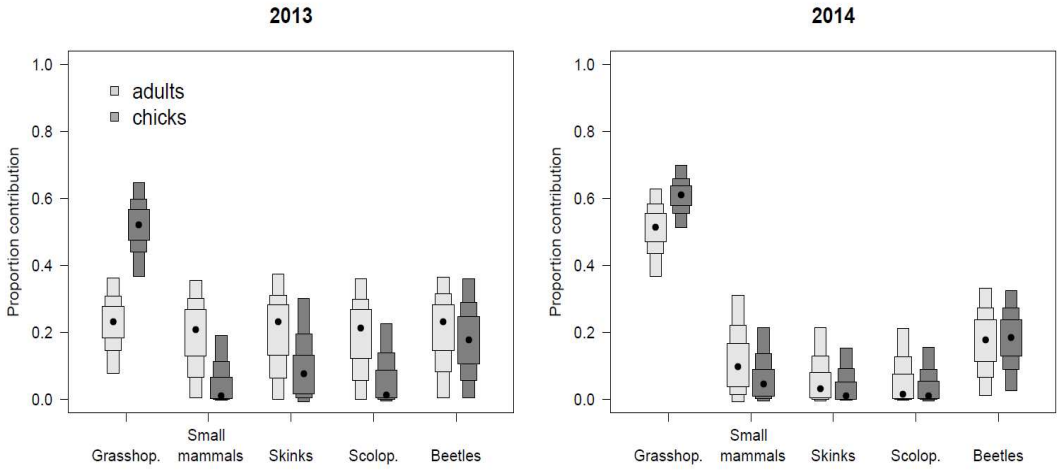

adults 2013
chicks 2013
adults 2014
chicks 2014
11
10
9
8
7
6
−26.0
−25.8
−25.6
−25.4
−25.2
−25.0
δ15N(‰)
δ13C(‰)

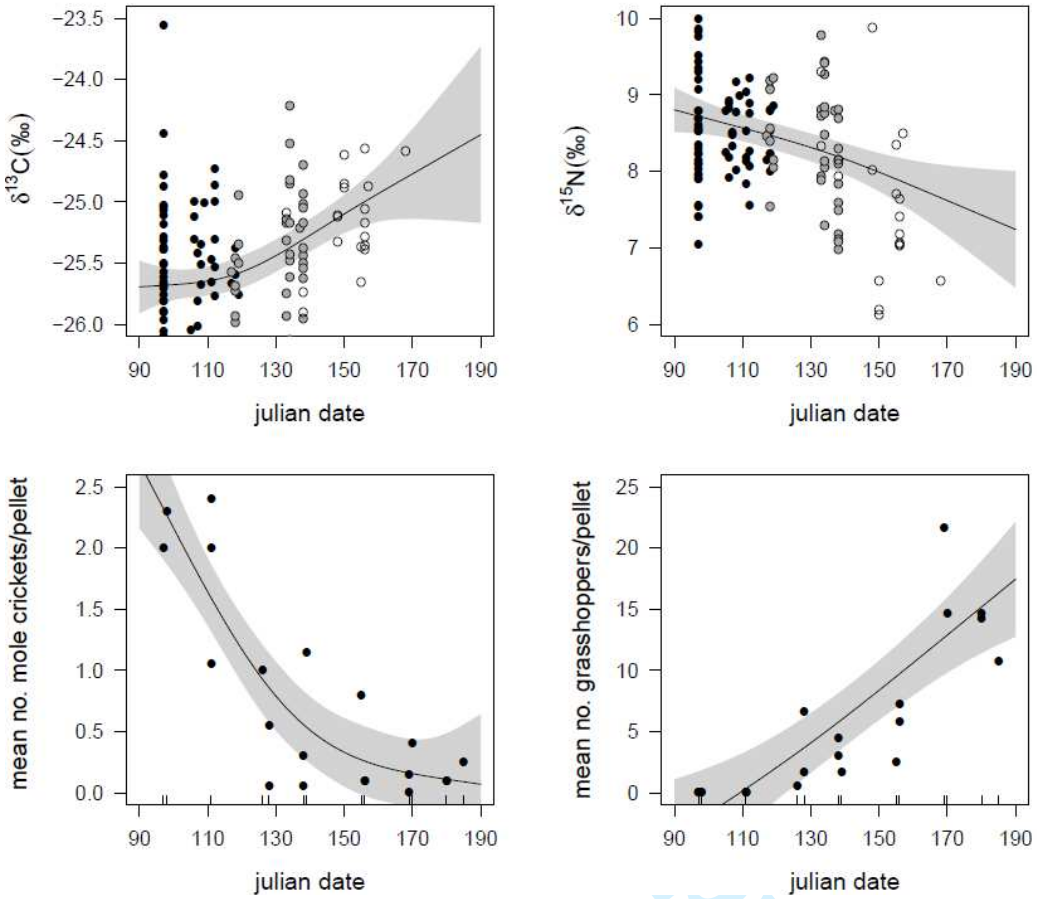
Figure 4



732 **Figure 5**

733

734



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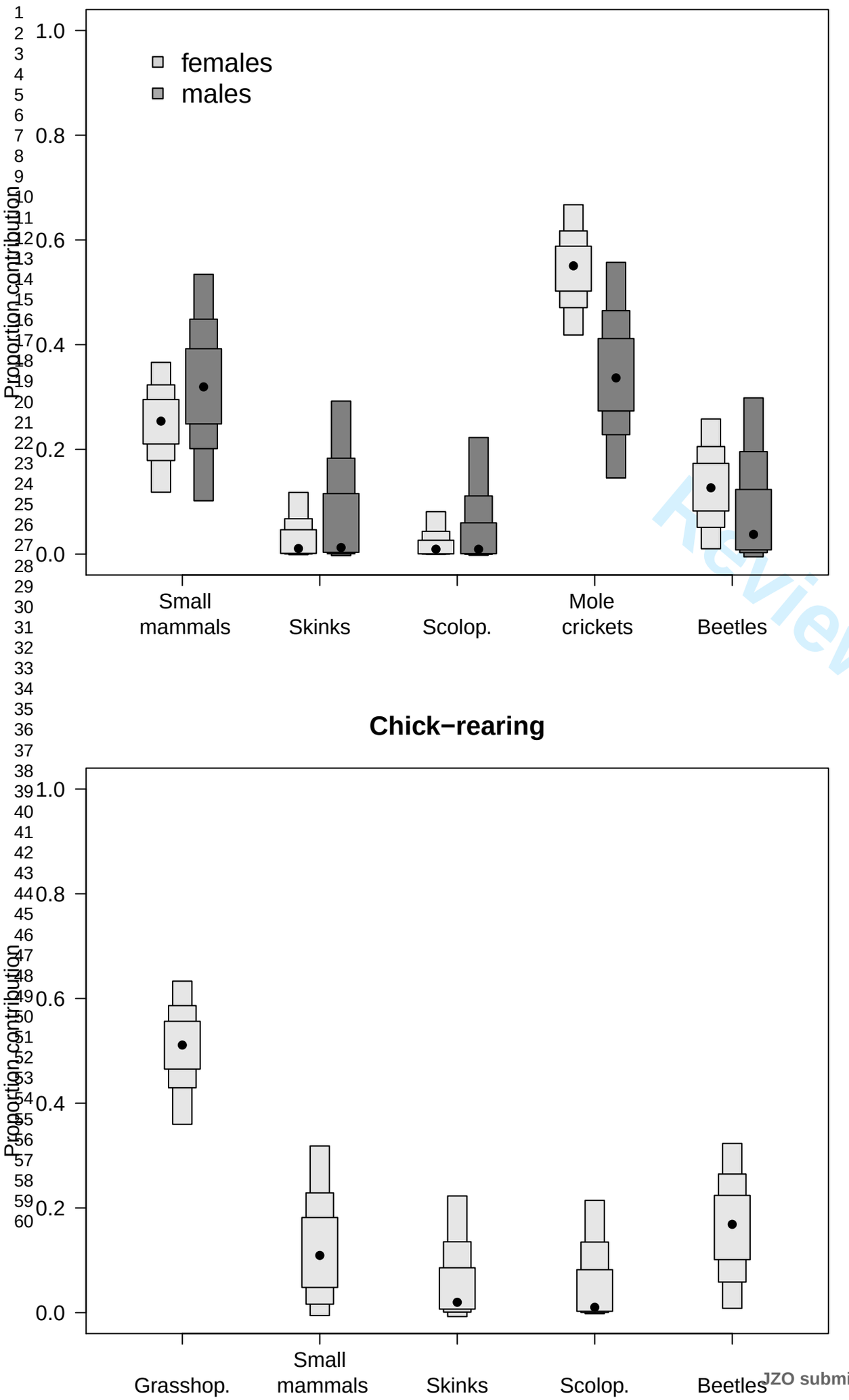
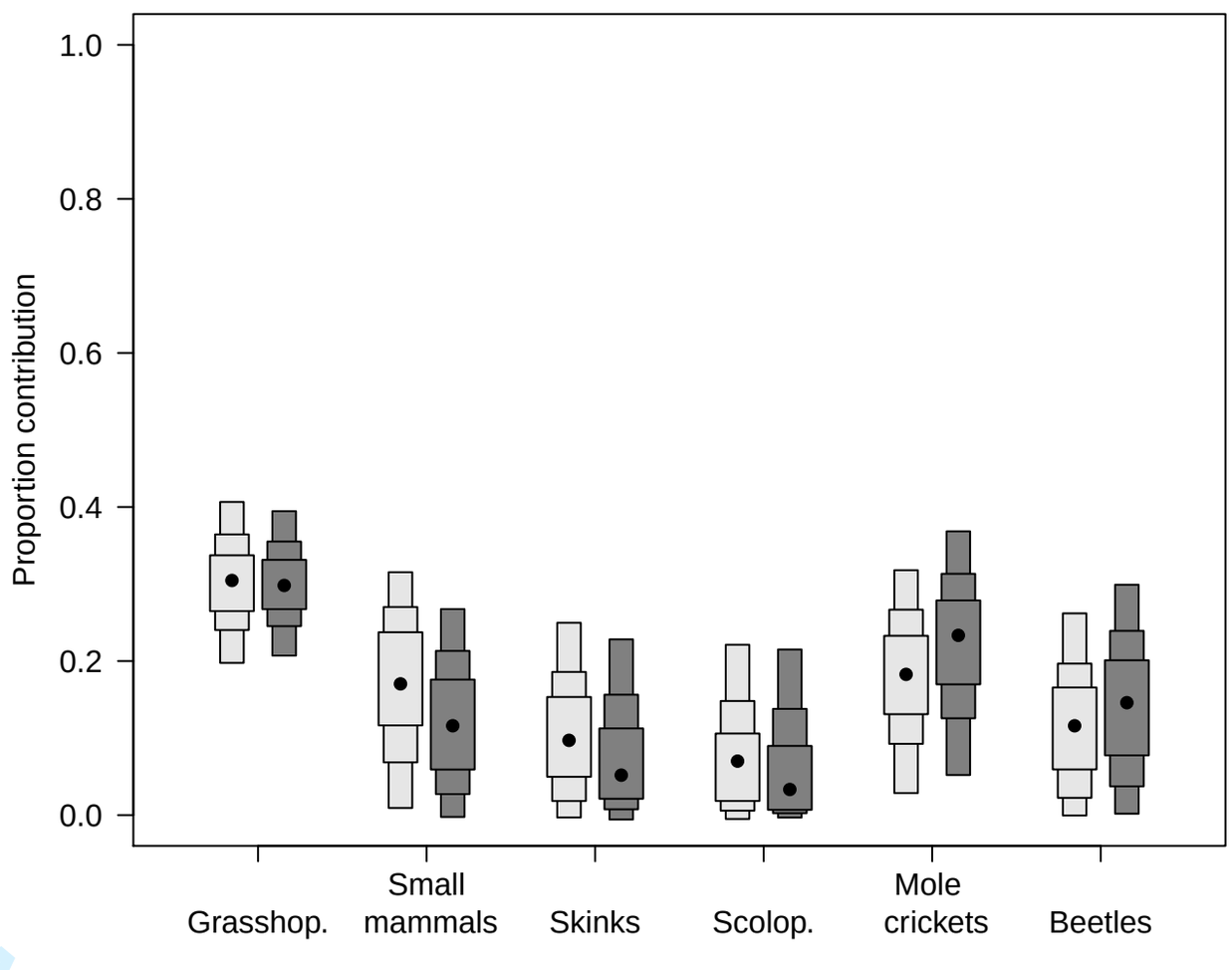
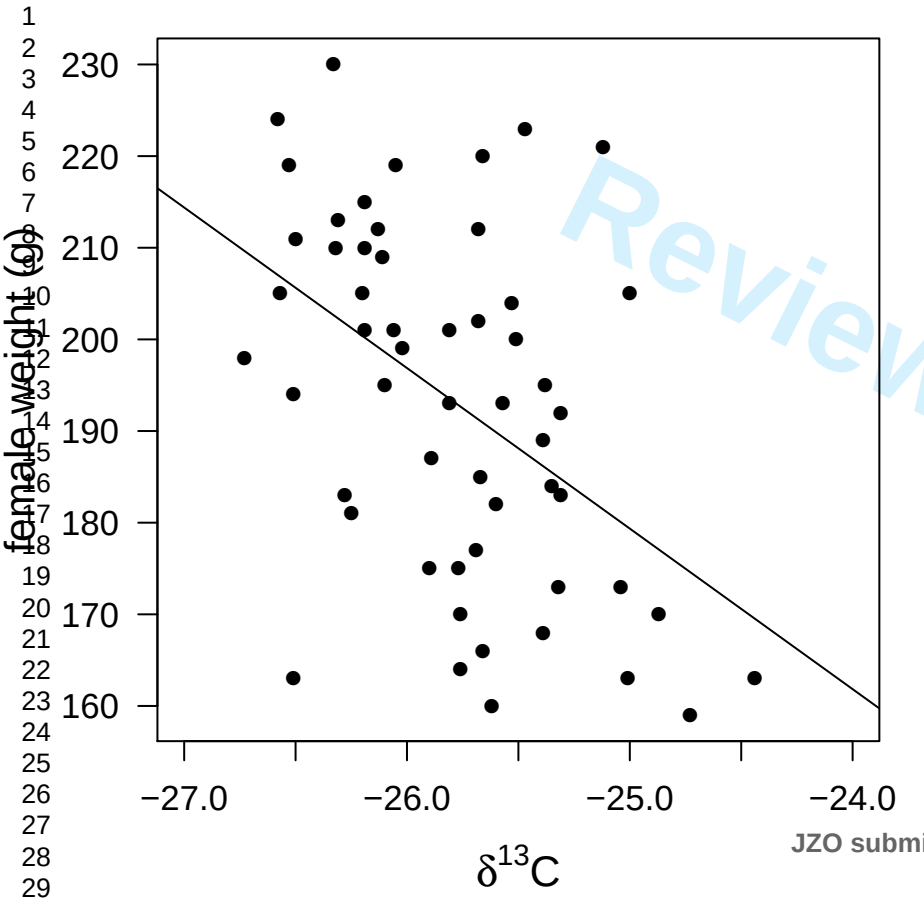
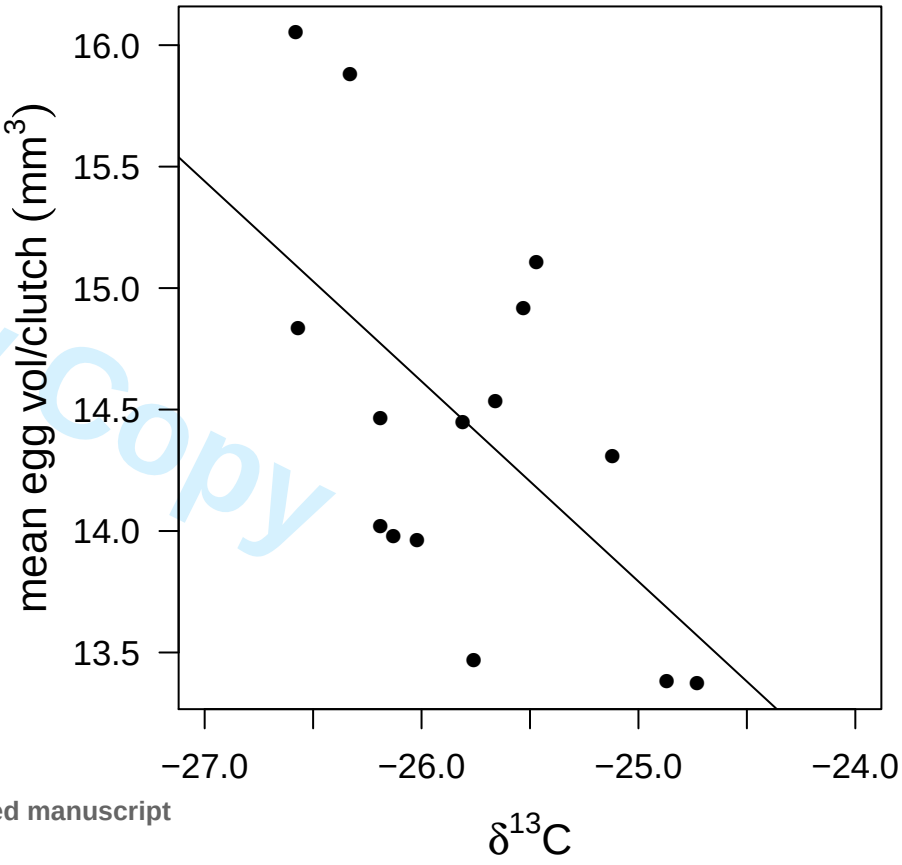
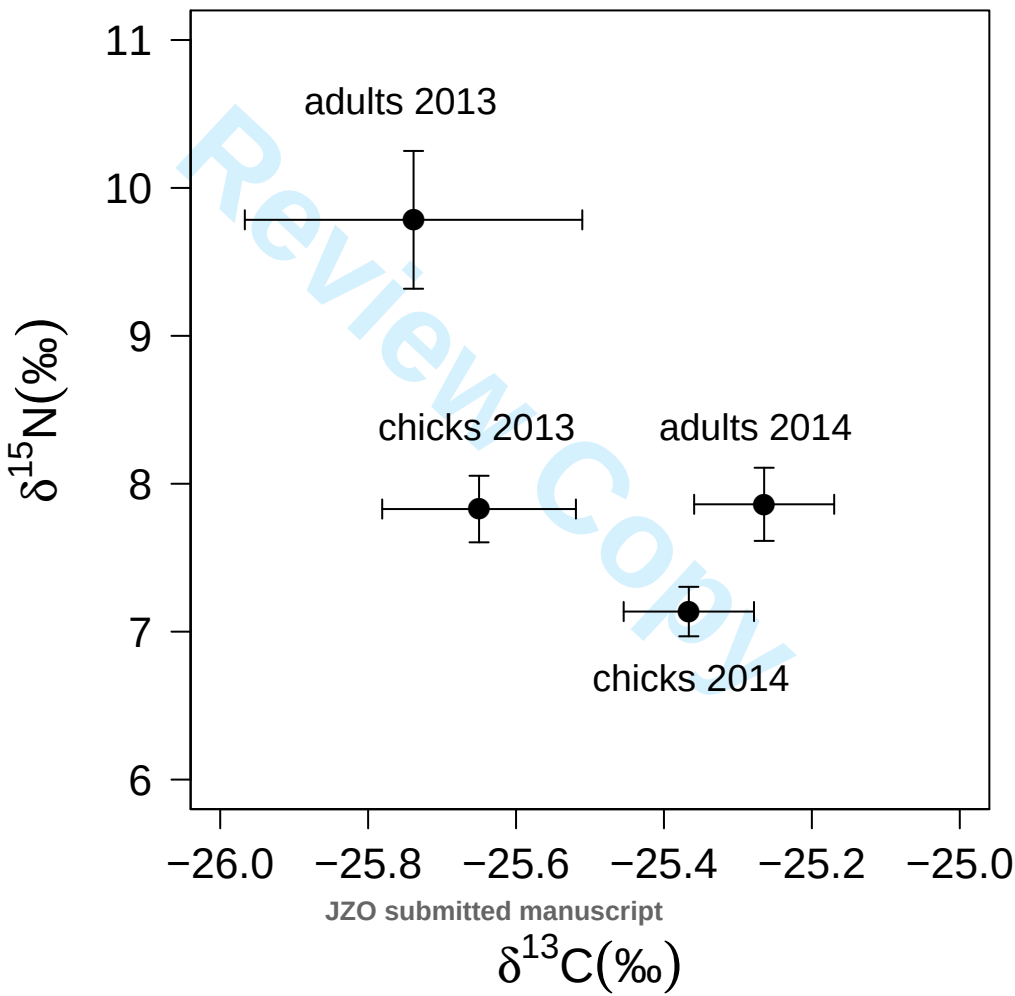
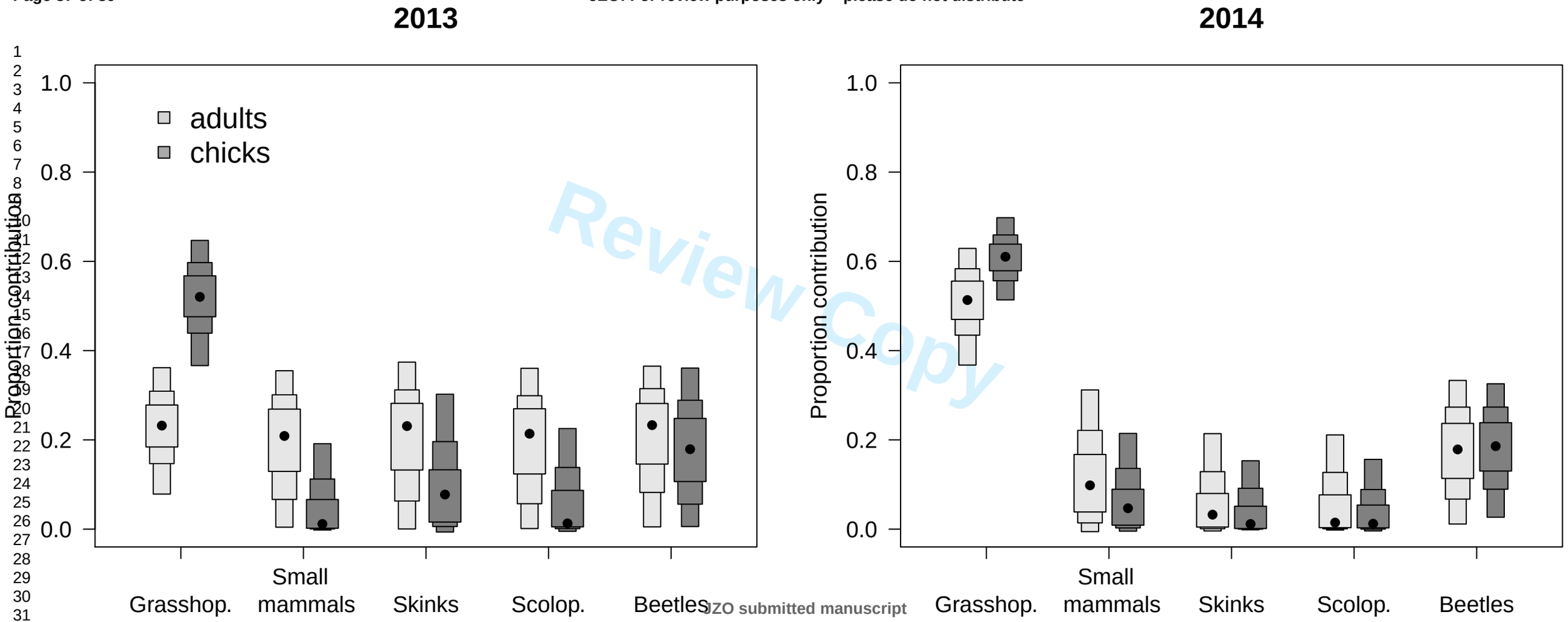


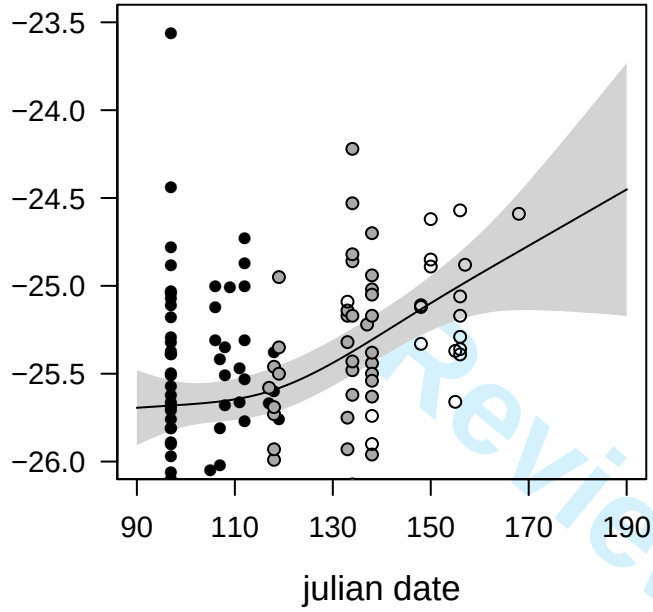


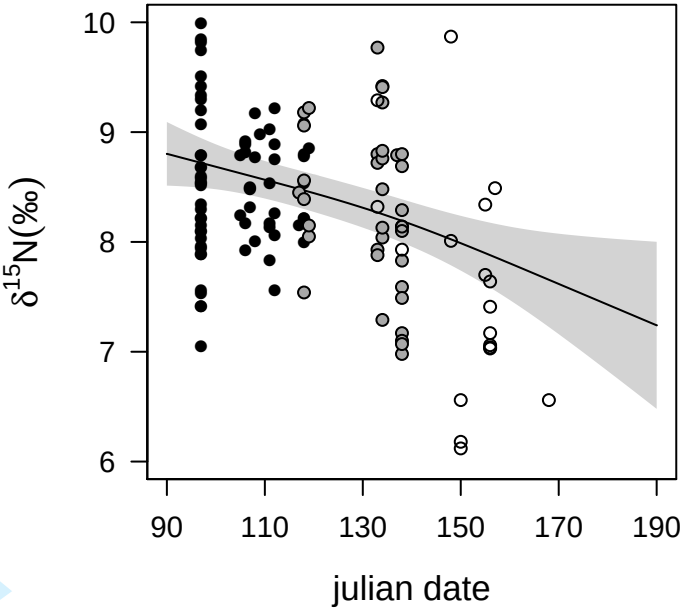


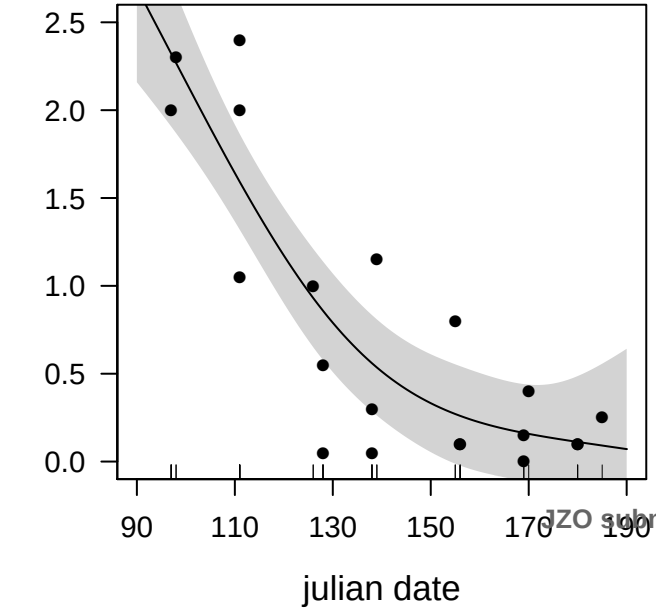


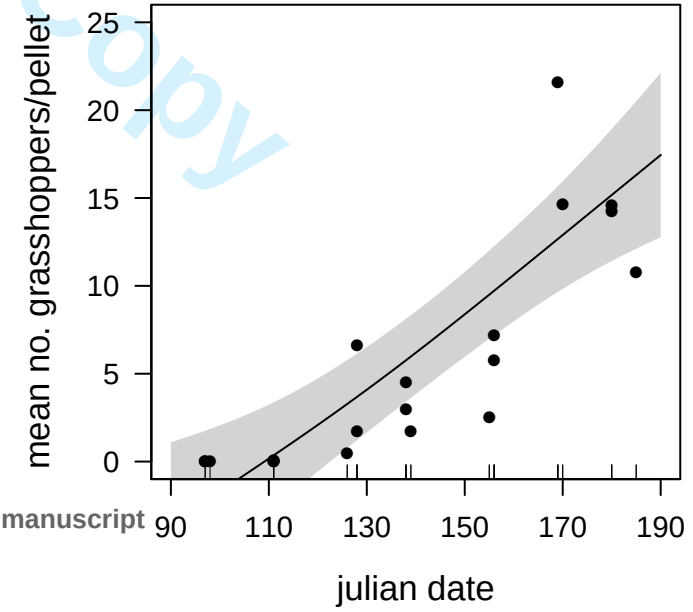

adults 2013
chicks 2013
adults 2014
chicks 2014
11
10
9
8
7
6
−26.0
−25.8
−25.6
−25.4
−25.2
−25.0
δ15N(‰)
δ13C(‰)











SUPPORTING INFORMATION

Appendix 1. List of published studies in which isotopic discrimination factors for bird species was assessed. Given the absence of previous studies experimentally determining discrimination factors in lesser kestrels (or in bird species with similar dietary composition) we estimated the mean (±SD) value of trophic discrimination factors for blood samples of several bird species reported in these studies.

Cherel, Y., Hobson, K. A. & Hassani, S. (2005). Isotopic discrimination between food and blood and feathers of captive penguins: implications for dietary studies in the wild. *Physiol. Biochem. Zool.* **78**, 106–115.

Evans-Ogden, L. J., Hobson, K. A. & Lank, D. B. (2004). Blood isotopic (d13C and d15N) turnover and diet-tissue fractionation factors in captive Dunlin (*Calidris alpina pacifica*). *Auk* **121**, 170–177.

Haramis, G. M., Jorde, D. G., Macko, S. A. & Walker, J. L. (2001). Stable isotope analysis of canvasback winter diet in Upper Chesapeake Bay. *Auk* **118**, 1008–1017.

Hobson, K. A. & Barlein, F. (2003). Isotopic fractionation and turnover in captive golden warblers (*Sylvia borin*): implications for delineating dietary and migratory association in wild passerines. *Can. J. Zool.* **81**, 1630–1635.

Pearson, S. F., Levey, D. J., Greenberg, C. H. & Martínez del Rio, C. (2003). Effects of elemental composition on the incorporation of dietary nitrogen and carbon isotopic signatures in an omnivorous songbird. *Oecologia* **135**, 516-523.