High trophic niche overlap in mixed-species colonies using artificial nests

JOÃO GAMEIRO[1*] (0000-0002-9871-9763), TERESA CATRY[2] (0000-0003-4047-9995), JOANA MARCELINO[3] (0000-0003-3404-5007), ALDINA M.A. FRANCO[4] (0000-0001-6055-7378), JORGE M. PALMEIRIM[1] (0000-0003-4734-8162), INÊS CATRY[5,6,7] (0000-0002-5593-5001)

[1]*cE3c – Centre for Ecology, Evolution and Environmental Changes, Departamento de Biologia Animal, Faculdade de Ciências, Universidade de Lisboa, 1749-016 Lisboa, Portugal*

[2]*Centro de Estudos do Ambiente e do Mar (CESAM), Departamento de Biologia Animal, Faculdade de Ciências da Universidade de Lisboa, 1749-016 Lisboa, Portugal*

[3]*Centre for Applied Ecology "Prof. Baeta Neves"/InBIO Associate Laboratory, Instituto Superior de Agronomia, Universidade de Lisboa, Tapada da Ajuda, 1349-017, Lisboa, Portugal.*

[4]*School of Environmental Sciences, University of East Anglia, NR4 7TJ Norwich, UK*

[5] *CIBIO/InBIO, Centro de Investigação em Biodiversidade e Recursos Genéticos, Laboratório Associado, Universidade do Porto, Campus Agrário de Vairão, Rua Padre Armando Quintas, 4485–601 Vairão, Portugal*

[6] *CIBIO/InBIO, Centro de Investigação em Biodiversidade e Recursos Genéticos, Laboratório Associado, Instituto Superior de Agronomia, Universidade de Lisboa, 1349-017 Lisbon, Portugal*

[7] *BIOPOLIS Program in Genomics, Biodiversity and Land Planning, CIBIO, Campus de Vairão, 4485-661 Vairão, Portugal*

Although successful at recovering endangered populations, conservation actions based on nest provisioning seldom consider how they shape the composition of communities and alter interspecific interactions. Specifically, the extent to which dietary overlap within these communities may affect the conservation of target species has rarely been assessed. In Southern Europe, large-scale nest-site provisioning aimed at recovering Lesser Kestrels *Falco naumanni* populations attracted non-target bird species, resulting in mixed breeding assemblages that might promote interspecific competition for resources during breeding. Here we used stable isotope analysis ($\delta^{15}N$ and $\delta^{13}C$) to assess inter- and intraspecific dietary segregation in these assemblages and investigate the mechanisms allowing species coexistence. We examined resource partitioning and trophic niche overlap among Lesser Kestrels, Common Kestrels *Falco tinnunculus*, European Rollers *Coracias garrulus*, Western Barn Owls *Tyto alba*, Little Owls *Athene noctua*, and Spotless Starlings *Sturnus unicolor*; and within species between parents and their offspring. Similar isotope ratios and highly overlapped niches, particularly among Lesser Kestrels, Rollers, and Starlings, suggest limited dietary segregation and use of similar prey. Within species, parent-offspring segregation was marked across all species. Our results indicate that species breeding in these assemblages occupy similar ecological niches, despite a potential increase in competition. High-resource availability in the area may permit coexistence but the viability of mixed-species groups may be compromised in areas with limited resources, which are predicted to expand with ongoing human and climate-induced changes. Conservation practices based on nest provisioning should consider the ecological niches of target and non-target species as well as their interactions.

Keywords: isotopic niche, dietary segregation, parent-offspring segregation, interspecific competition, mixed-species colonies, community

Human-induced changes in the environment shape the distribution of many species by restricting their access to resources such as food or nest-sites (Wiegand *et al*. 2005; Teckentrup *et al*. 2019). Ongoing biodiversity loss has prompted conservation projects including provisioning of artificial nest-sites to halt species decline in many taxa and regions (Harper *et al*. 2005; Catry *et al*. 2009; Olah *et al*. 2014; Sutherland *et al*. 2018), further re-shaping the nesting landscape and altering the composition and structure of communities (Duckworth *et al*. 2017; Catry & Catry 2019). Nest-site provisioning may modify interspecific interactions and trophic dynamics (Duckworth *et al*. 2017; Catry & Catry 2019), but their consequences can be subtle and potentially overlooked by researchers or conservation managers (Mainwaring *et al*. 2015).

According to niche theory and the competitive exclusion principle, species with similar niches, likely to compete with one another, are not expected to coexist (Hardin 1960; Pianka 1981). If a new and artificial nesting landscape promotes the aggregation of potential competitor species, artificial nest-sites may turn into ecological traps by reducing long-term individual fitness through a reduction of resource availability or increased interspecific aggressive behaviours (Kappeler *et al*. 2015; Catry & Catry 2019).

To avoid competition, especially when resources are limited, coexisting species can segregate in at least one of three main axes: space, time, and diet (Pianka 1981). Segregation in spatio-temporal habitat utilisation occurs when species forage in different locations (e.g. insectivorous desert bats, Razgour *et al*. 2011), different periods of the day (e.g. African carnivore communities, Schuette *et al*. 2013), or when they have different breeding phenologies (e.g. sympatric penguin species, Clewlow *et al*. 2019). Dietary segregation occurs when species reduce their dietary niche overlap by foraging for alternative food resources or similar items with different sizes (Macarthur & Pianka 1966; Jenkins & Davoren 2020; Mansor *et al*. 2021). Species may also contract their dietary niches to focus on certain prey types that become temporarily super-abundant, which increases the dietary niche overlap among species without increasing competition (Forero *et al*. 2004; Charter *et al*. 2018; Denhard *et al*. 2020). Dietary niche segregation can also occur within species to reduce intraspecific competition, through sexual or parent-offspring segregation, especially when the potential for competitive interactions is high, such as for central place foragers during reproduction (Orłowski *et al*. 2014; Catry *et al*. 2016a; Reisinger *et al*. 2020).

Here, we investigate dietary niche overlap in a multi-species bird assemblage created by large-scale nest-site provisioning aimed at recovering the colonial Lesser Kestrel *Falco naumanni* in southern Portugal (Catry *et al*. 2009). In a treeless landscape with few nesting opportunities, many bird species took advantage of these artificial breeding structures – some with more than 80 cavities, resulting in mixed-species colonies (Catry & Catry 2019). These include Lesser Kestrels, Common Kestrels *Falco tinnunculus*, European Rollers *Coracias garrulus*, Western Barn Owls *Tyto alba*, Little Owls *Athene noctua*, Western Jackdaws *Corvus monedula*, Spotless Starling *Sturnus unicolor*, and Feral Pigeons *Columba livia* (Catry & Catry 2019). Excluding Feral Pigeons and Western Jackdaws, the dietary habits of these species, including mostly large arthropods and small rodents, suggest that their use of trophic resources may overlap to a large extent (Jaksić *et al*. 1982; Motis *et al*. 1997; Tomé *et al*. 2008; Catry *et al*. 2016a; Orihuela-Torres *et al*. 2017; Catry *et al*. 2019), especially during the breeding season, when competition for food should peak as parents need to obtain resources to fulfil their own energy requirements and those of their offspring (Orłowski *et al*. 2014; Antón-Tello *et al*. 2021). High densities of breeding pairs could lead to prey depletion in the vicinity of the colonies, further increasing inter and intraspecific competition (sensu Ashmole’s halo; Birt *et al*. 1987; Dehnhard *et al*. 2020; Jenkins and Davoren, 2020).

We used stable isotope analysis (SIA) to investigate inter and intraspecific overlap in the diet of six bird species breeding in mixed colonies installed in artificial breeding structures to better understand the mechanisms allowing their coexistence. Specifically, using stable isotope ratios of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$), we aimed to: (1) compare isotopic niche breadth and overlap among the species and (2) assess parent-offspring diet segregation within each species. We sampled feathers from nestlings and blood plasma from parent-offspring pairs during the breeding season, providing detailed insight into the dietary resources used by these species. We predict species with similar diets and similar trophic positions to show higher dietary overlap, while species at the top and bottom ends of the trophic spectrum to be more segregated from the rest of the assemblage. We consider the extent to which dietary overlap and niche segregation among potential competitors may affect the conservation of Lesser Kestrels.

2. Methods:

2.1 Study area and study species

This study was carried out in the Castro Verde Special Protection Area (SPA, Natura 2000), Southeast Portugal (37º41'N, 8º05'W). At about 85,000 ha, this is the main area of agro-steppe in Portugal – a semi-natural habitat created by low intensity farming practices – and one of the main strongholds for several threatened farmland bird species in Western Europe (Moreira *et al*. 2007; BirdLife International 2021). Land use within the study area has remained relatively stable in recent decades, in part due to the implementation of agri-environmental policy schemes and funding mechanisms that ensure high-quality foraging habitat for many farmland birds (Catry *et al*., 2013; Silva *et al*., 2018). Although potential changes in prey availability were not assessed across the study period, habitat stability suggests no major changes in prey composition and abundance. Mixed-species colonies are mostly found in artificial nesting structures provided by LIFE conservation projects target at

Lesser Kestrels during the early 2000s (Catry *et al*. 2009), and to a lesser extent in abandoned farmland buildings, with nests inside wall cavities or under roof tiles. Colonies have been monitored annually and their composition (number of pairs of each species) has remained similar in recent years. Within these breeding assemblages, we focused on species that prey mainly on small vertebrates and arthropods, as these have higher potential for trophic niche overlap: Western Barn Owl, Little Owl, Common Kestrel, Lesser Kestrel, European Roller and Spotless Starling. Feral Pigeons and Western Jackdaws are mostly herbivorous and include a low proportion of animal prey in their diet (Murton & Westwood 1966; Högstedt 1980; Soler *et al*. 1990), hence dietary overlap with the carnivorous or insectivorous species is expected to be very low. Furthermore, because of incomplete diet information, we would not be able to correctly interpret their isotopic niches and determine the diet overlap with the remaining species. Feral Pigeons and Western Jackdaws were, therefore, excluded from this study.

2.2. Sample collection

Feather sampling was carried out during the breeding seasons of 2014, 2016 and 2018 (Table 1). Nestlings of Western Barn Owls (n=12), Little Owls (n=19), Common Kestrels (n=33), Lesser Kestrels (n=36), European Rollers (n=36), and Spotless Starlings (n=12) were sampled in their nests at the age of 3-4 weeks, when breast feathers are well developed. Approximately 3-5 breast feathers were plucked from one nestling per nest (keeping the feather base intact) and stored in separate plastic zip bags. Blood sampling of parent-offspring pairs – one parent and one nestling – of Little Owls (n=7), Common Kestrels (n=8), Lesser Kestrels (n=11), European Rollers (n=6), and Spotless Starlings (n=6) was carried out during the 2019 breeding season (Table 1). Parents and their offspring were sampled within a narrow temporal window (6.9 ± 6.1 days) at their nests. Approximately 150 µL of blood was collected from the brachial vein and preserved in separate vials. The blood was centrifuged for 10 min at 3400 rev/min within 3 hours of collection, to separate plasma from red blood cells, and plasma samples were frozen before being further prepared for stable isotope analysis (red blood cells were not analysed). We were unable to sample enough Western Barn Owl adults, and so parent-offspring segregation was not investigated for this species.

Additionally, to aid the interpretation of stable isotope data, we sampled key prey types of each species in mixed-species colonies throughout the 2018 breeding season (Table 2). Prey species were identified and collected inside or near the nests and were kept frozen until processed for isotope analysis. All work involving bird handling and sample collection was approved by the Instituto de Conservação da Natureza e Florestas (ICNF, permits 547/2018, 427/2019).

2.3. Stable isotope analysis

Stable isotope ratios have been widely used as dietary tracers to assess different habitats and dietary resources used by consumers, providing a proxy for species or individual ecological niches using minimally invasive methods (Forero *et al*. 2004; Catry *et al*. 2019; Dehnhard *et al*. 2020). Stable isotope analysis of nitrogen ($\delta^{15}N$) and carbon ($\delta^{13}C$) provides information on the trophic position of an organism in the food chain and on habitat use, respectively, allowing researchers to simultaneously examine dietary niche segregation/overlap of multiple species or individuals within assemblages and make inference about competition for resources (Inger & Bearhop 2008; Alonso *et al*. 2012; Catry *et al*. 2016b; Reisinger *et al*. 2020; Jenkins and Davoren, 2020). Stable isotope analysis also provides information on the dietary niche at multiple temporal scales depending on the tissue sampled, as isotope ratios reflect the diet during which that tissue was metabolically active.

Here, we use feathers from nestlings of the six study species to examine isotopic breadth and overlap among species, as they offer information for the period during which the feather was being formed, adequately characterising the diet of the nestlings during the breeding season for the feather growing period (Inger & Bearhop 2008; Weiss *et al*. 2009). Feathers were washed in double baths of 0.25N sodium hydroxide solution alternated with baths of double distilled water to remove any surface contaminants, and were then air dried and then cut into small fragments (Catry *et al*. 2008). To investigate parent-offspring dietary segregation, we used blood plasma. Blood plasma has a higher turnover rate than feathers, reflecting the averaged diet over shorter periods (days), and thus allows for a temporally aligned comparison between parent and offspring signatures (Inger & Bearhop 2008; Reisinger *et al*. 2020). Blood plasma from adult birds was additionally used to infer niche overlap between species in addition to nestling feathers. Information from nestling plasma was only used to examine parent-offspring segregation, as it does not add information to that obtained with nestling feathers (sample size was smaller and restricted to one year, and plasma reflects information on the birds' diet over a much shorter period). Plasma samples of birds and soft tissues (muscle) of prey were dried in an oven at 60°C for 48h and then homogenised to a powder. Prey samples were then processed for lipid extraction by immersion in a 2:1 chloroform/methanol solution with a solvent volume three to five times larger than the sample volume (Logan *et al*. 2008). Samples were then mixed for 30s, left undisturbed for ca. 30 min, further centrifuged for 10 min at 3400 rev/min, and the supernatant containing solvent and lipids was removed. This process was repeated at least three times (until the solvent was clear) and samples were then re-dried at 60ºC for 24h to remove any remaining solvent. Between 0.8 and 1.2 mg of each sample (feathers, plasma, and prey) were weighted and stored in tin cups for stable carbon and nitrogen isotope assays. Isotopic ratios were determined by continuous-flow isotope ratio mass spectrometry at the Stable Isotopes and

Instrumental Analysis facility of the faculty of Sciences, University of Lisbon. Results are presented conventionally as δ values in parts per thousand (‰) relative to the IAEA CH7 and Glucose BCR for $\delta^{13}C$, and USGS25 and USGS35 for $\delta^{15}N$. The precision of the isotope ratio analysis, calculated using values from six to nine replicates of laboratory standard material (casein) interspersed among samples in every bath analysis, was 0.04-0.05‰ and 0.02-0.04‰ for $\delta^{13}C$ and $\delta^{15}N$, respectively.

2.4 Data analysis

Feather isotopic signatures for each species from all years were pooled together as the relative isotopic position of each species remained similar (Table 1; Fig. S1).

Differences in isotopic signatures among species were examined using pairwise multivariate analyses of variance performed on nitrogen and carbon simultaneously. Then analyses of variance were performed on carbon and nitrogen separately, followed by pairwise comparisons between each pair of species. Each set of analyses was performed for both nestling feathers and adult plasma. Non-parametric tests were used for nestlings (PERMANOVA, Kruskall-Wallis, and Wilcoxon rank sum tests), and parametric tests for adults (MANOVA, ANOVA, and Tukey tests), after assessing data normality.

Prey signatures were only used to broadly characterise the birds' diet and help interpret differences and similarities in the isotopic niches of the different consumer species, i.e., without quantitatively assessing the contribution of each prey type (Jackson & Parnell 2020). Prey items with similar isotopic signals were pooled within each taxonomic order (SIA group, Table 2), and then plotted in the isotopic biplot graph to aid the interpretation of consumer signatures using trophic enrichment factors (TEF). Due to lack of reported discrimination factors for all consumer species, we used the mean (± SD) value of trophic discrimination factors for feather and plasma samples of other bird species reported in published studies reviewed by Caut *et al*. (2009; feathers: $\delta^{13}C = 2.16 \pm 1.53$, $\delta^{15}N = 2.84 \pm 1.14$; plasma: $\delta^{13}C = -0.08 \pm 0.85$, $\delta^{15}N = 2.82 \pm 0.31$).

To measure isotopic niche breadth of each species and estimate the degree of niche overlap between species, corrected and Bayesian standard ellipse areas (SEA_C and SEA_B, respectively) were calculated using the SIBER package for R (Jackson & Parnell 2020). SEA_B were used to quantitatively compare niche breadth among species, using 1 000 000 iterations, three chains, a burn-in of 1000 and thinning of 10, using a vague normal prior (Jackson & Parnell 2020). Overlap among SEAc for all pairs of species and for parents and offspring within each species were estimated to reflect the degree of trophic resources shared. For each species (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 $SEA_{[i]}$ ($A_{[i]}$), expressed as a proportion ($Ov_{]i]} = A_{[I,j]}/A_{[i]}$; Catry *et al*. 2016b). Average overlap per species was estimated as the average of all overlaps involving that species. Additionally, to evaluate parent-offspring segregation within each species, differences in Euclidean distances between the centroids of parents and offspring were calculated and tested using a residual permutation procedure based on Turner *et al*. (2010). Afterward, differences in $\delta^{15}N$ and $\delta^{13}C$ between parents and offspring were compared with t-tests or Mann-Whitney U tests for parametric and non-parametric data, respectively. Assumptions of each test were met prior analysis. All analyses were conducted in the R statistical environment (R Development Core Team, 2016).

3. Results

3.1 Stable isotope signatures

Feathers were collected from 148 nestlings, and plasma samples were taken from 38 parent-offspring pairs of six different species; mean (± SD) stable isotope signatures ($\delta^{13}C$, $\delta^{15}N$) for each species are presented in Table 1 and Figure 1. A PERMANOVA on $\delta^{13}C$ and $\delta^{15}N$ of nestling feathers revealed differences among species ($F_{(5,142)} = 23.618$, $P = 0.001$), both in $\delta^{13}C$ and in $\delta^{15}N$ (Kruskal-Wallis tests $\delta^{13}C$: $\chi^2_{(5,142)} = 15.948$, $P = 0.007$; $\delta^{15}N$: $\chi^2_{(5,142)} = 87.323$, $P < 0.001$). Post-hoc tests indicated that $\delta^{13}C$ only differed between Lesser Kestrels and European Rollers ($P = 0.02$). Western Barn Owls and Little Owls fed at the highest trophic level (most enriched $\delta^{15}N$ values) of the community, significantly higher than all other species (all $P < 0.005$). Common Kestrels had more enriched $\delta^{15}N$ values than Lesser Kestrels and European Rollers, (both $P < 0.001$), but similar values to Spotless Starlings ($P = 0.257$). Spotless Starlings also had more enriched $\delta^{15}N$ than Lesser Kestrels ($P = 0.016$) and European Rollers ($P < 0.001$) and Lesser Kestrels had higher values than European Rollers ($P = 0.012$). Regarding the adult community, blood isotopic signatures were only different among species for $\delta^{15}N$ values (MANOVA: $F_{(4,33)} = 4.97$, $P < 0.001$;ANOVA $\delta^{13}C$: $F_{(4,33)} = 2.038$, $P = 0.112$; $\delta^{15}N$: $F_{(4,33)} = 10$, $P < 0.001$). The trophic rank of adults was similar to that of nestlings. Little Owls and Common Kestrels had the highest $\delta^{15}N$ values (adult Western Barn Owls were not sampled), with Little Owls being in a significantly higher position than Lesser Kestrels, European Rollers, and Spotless Starlings (all $P < 0.001$), while Common Kestrels had only significantly higher $\delta^{15}N$ values than Spotless Starlings (*P-values*: Lesser Kestrel = 0.061, European Roller = 0.068, Spotless Starling = 0.030). Adult Lesser Kestrels, European Rollers and Spotless Starlings had similar $\delta^{15}N$ values (all $P > 0.900$; Fig. 1B). In short, trophic position (according to $\delta^{15}N$ values) of nestlings and adults in the community was Western Barn Owl = Little Owl > Common Kestrel = Spotless Starling > Lesser Kestrel > European Roller; and Little Owl = Common Kestrel > Spotless Starling = Lesser Kestrel = European Roller; respectively (Fig.1 and 2).

Isotopic signatures of the main prey items (corrected by TEF) are given in Figure 1. The 10 potential prey groups comprise a broad isotopic space, with mean $\delta^{13}C$ values ranging from -27.3 ± 0.6 to 23.2 ± 0.3 and mean $\delta^{15}N$ from 3.4 ± 1.5 to 10.9 ± 3.0 (Table 2; Fig. 1). The relative trophic position of the six species and their relation to prey suggest that Common Kestrels, Lesser Kestrels, European Rollers, and Spotless Starlings feed their offspring mostly with Orthoptera and to a lesser extent with Coleoptera and vertebrates, while the opposite occurs in Western Barn Owls and Little Owls (Fig. 1A). Regarding adults, Little Owls and Common Kestrels had closer signatures to vertebrates, while Lesser Kestrels, European Rollers, and Spotless Starlings maintained a lower trophic position.

3.2 Interspecific isotopic niche overlap

As observed for the consumers' isotopic space described above, differences in trophic niches were found mainly between species likely including vertebrates in their diet, i.e., Western Barn Owls and Little Owls, and those preying mostly on arthropods, such as Lesser Kestrels, European Rollers, and Spotless Starlings; with Common Kestrels in an intermediate position (Fig. 2). Amongst nestlings, Little Owls had the widest niche breadth (SEA_B = 4.352; Table 3), only slightly overlapping with Common Kestrels and Spotless Starlings (23% and 12%, respectively; Table S1) but fully overlapping with Western Barn Owls (SEA_B = 0.755; Table 3; Fig. 2A). Common Kestrels and Spotless Starlings also showed a high overlap with each other (63% and 75%) as well as with Little Owls (59% and 37% respectively; Table S1). Lesser Kestrel nestlings had the smallest niche breadth (SEA_B = 0.505; Table 3) which highly overlapped with the dietary niche of European Rollers, Common Kestrels, and Spotless Starlings (60%, 40% and 60%, respectively; Table S1). Amongst adults, Lesser Kestrels, European Rollers, and Spotless Starlings showed high niche overlap, while Common Kestrels and Little Owls were clearly segregated (Fig. 2B; Table S1).

3.3 Parent-offspring segregation

Differences between adults and nestlings were found in δ^{15}N values for every species except Little Owls (Little Owl: $w_{(12)} = 38$, $P = 0.097$; Common Kestrel: $w_{(14)} = 68$, $P = 0.001$; Lesser Kestrel $w_{(20)} = 107$, $P = 0.001$; European Roller: $t_{(10)} = 2.753$, $P = 0.025$; Spotless Starling: $w_{(10)} = 34$, $P = 0.009$). No differences in δ^{13}C values were found for any species (Little Owl: $t_{(12)} = -1.961$, $P = 0.075$; Common Kestrel: $t_{(14)} = 0.381$, $P = 0.709$; Lesser Kestrel: $t_{(20)} = 1.256$, $P = 0.228$; European Roller: $t_{(10)} = 0.115$, $P = 0.115$; Spotless Starling: $t_{(10)} = -0.083$, $P = 0.936$). Despite this, Euclidean distance between centroid location of adults and nestlings was significantly different in all species (all $P < 0.01$; Table S2), indicating a high parent-offspring segregation in all of them (Fig. 3).

4. Discussion

Identifying the dietary niche of species within assemblages is crucial to understand the mechanisms allowing the coexistence of potential competitors in a specific area and design adequate conservation management actions for mixed-species colonies. Here, we used carbon and nitrogen stable isotope ratios from nestlings and from parent-offspring pairs to provide a detailed assessment of the dietary resources used by a group of six bird species breeding in dense mixed-species colonies established as the result of an extensive nest-provisioning program (Catry *et al*. 2009; Catry & Catry, 2019). Overall, our results show a high intraspecific (parent-offspring) but low interspecific dietary segregation in these assemblages, suggesting a high potential for interspecific competition for resources during the breeding season.

4.1 Interspecific overlap in isotopic niches

Identical isotopic signatures and highly overlapping niches suggest that species breeding in these assemblages rely on similar prey to feed their offspring. As reported in previous studies, the low δ^{15}N isotopic signatures of Lesser Kestrel and European Roller nestlings likely reflect a diet based on Orthoptera such as grasshoppers and bush-crickets (> 50% and 70% of all prey delivered to Lesser Kestrel and European Roller nestlings in the study area, respectively; Catry *et al*. 2016a, 2019). Thus, the high overlap observed between the two species was expected. Little Owl nestlings had the largest isotopic niche breadth in our study, overlapping with Common Kestrels but segregating from Lesser Kestrels and European Rollers. Common Kestrels and Little Owls have more generalist diets that include vertebrates but may expand their niche to consume more abundant prey such as beetles and grasshoppers, especially during chick rearing (Tomé *et al*. 2008; Orihuela-Torres *et al*. 2017). Remains of birds, amphibians, reptiles, and small mammals, but also of Orthoptera, were frequently found in Little Owl and Common Kestrel nests in the study area during chick rearing, confirming their eclectic diet. Nestlings of Western Barn Owls had the most enriched nitrogen signatures of the assemblage, but their dietary niche breadth suggests a more specialised, vertebrated-based diet (Jacksić *et al*. 1982; Riegert *et al*. 2021; authors pers.obs), segregating from all other species except Little Owls. Although there is no previous information on Spotless Starling diet in our study area, in general they rely mostly on small insects such as hoverflies, ants, and larvae of Coleoptera and Lepidoptera (Motis *et al*. 1997), which should give them a distinct isotope signature. However, we often observed Spotless Starlings feeding their offspring with grasshoppers, suggesting a similar diet and potentially explaining the observed niche overlap with the remaining community.

Isotopic characterisation of the adult community indicated a similar trophic rank, although the information retrieved from adult plasma is probably not representative of the whole breeding season, and results are based on few individuals in a single year. Adult Lesser

Kestrels and European Rollers showed a high overlap with Spotless Starlings, suggesting similar diets, while Common Kestrels and Little Owls had more enriched $\delta^{15}N$ values, likely reflecting the consumption of vertebrates.

4.2 Parent-offspring dietary segregation

Despite the lack of significant differences when comparing $\delta^{15}N$ and $\delta^{13}C$ values between Little Owl adults and nestlings separately, differences in Euclidean distance suggests that all species studied in these mixed colonies (including Little Owls) exhibited clear parent-offspring dietary segregation during the chick-rearing period. During a season of high energy demand, parents are expected to feed their offspring with high energy items, keeping less profitable resources for themselves (Orłowski *et al*. 2014). While this was already reported for Lesser Kestrels and European Rollers (Catry *et al*. 2016a, 2019), we could not find studies addressing parent-offspring segregation in the other studied species. This study reveals that Little Owls, Common Kestrels and Spotless Starlings also exhibit marked parent-offspring segregation in trophic resources, reinforcing that this may be a common evolutionary strategy to avoid intraspecific competition.

4.3 Niche theory applied to mixed-species colonies in an artificial nesting landscape

If on one hand the competitive pressure among species breeding together in dense aggregations can be alleviated through parent-offspring partitioning in their food resources, on the other hand the low dietary segregation among species can lead to an increase of interspecific competitive interactions (Catry & Catry 2019).

These results raise questions as to the mechanisms allowing species coexistence. According to ecological niche theory, species with high dietary overlap may still partition their niche and avoid interspecific competition through spatial or temporal segregation in habitat utilisation (Pianka 1981; Reisinger *et al*. 2020). In the mixed-species colonies studied, there is no evidence for temporal or spatial segregation, as the breeding phenology and chick rearing period of all species coincided, and as central-place foragers, all species hunted in the vicinity of the colonies (Casagrande *et al*. 2008; Catry *et al*. 2012; Šalek & Lövy 2012; Catry *et al*. 2017a). However, different species could still select different habitats or have hunting strategies that would foster niche segregation, an aspect that should be further investigated.

Alternatively, high niche overlap among sympatric species may reflect high availability of resources (Pianka 1981; Charter *et al*. 2018; Jenkins & Davoren 2020;). In the Castro Verde SPA, traditional farming practices associated with agro-steppes are maintained through specific agri-environmental policy schemes, ensuring high-quality foraging habitats for many farmland species (Catry *et al*. 2013, 2017a; Silva *et al*. 2018). Large areas of low-intensity grazed fallows support a high abundance of Orthoptera, and the chick-rearing period of Lesser Kestrels and European Rollers (and likely of the whole species assemblage except Western Barn Owls) coincides with the peak abundance of grasshoppers (Catry *et al*. 2016a, 2017b, 2019). From mid-May, the abrupt increase in grasshopper availability (Catry *et al*. 2017b), may allow species to contract their dietary niche to focus on temporarily super-abundant prey, increasing their overlap without necessarily increasing interspecific competition (Forero *et al*. 2004; Jenkins & Davoren 2020). The lack of differences in productivity between European Rollers breeding solitary and in mixed-species colonies, or between Lesser Kestrels in small or large colonies (Table S3), suggests that prey depletion around colonies in our study area is unlikely and that food availability is high enough to allow species with similar niches to coexist. This also suggest the study area might be able to support additional colonies, but this could imply other conservation implications, such as increasing these species' reliance on artificial nests and the financial costs associated with it (Gameiro et al. 2020).

While we found high trophic overlap among species within the studied community, we lack evidence to understand whether this may constrain the coexistence of these mixed-species colonies under some circumstances, an issue that is of particular importance for species of conservation concern, such as Lesser Kestrel and European Roller. Future studies could address how species coexist in areas with low resource availability, which are predicted to increase with ongoing human and climate-induced habitat changes (Tscharntke *et al*. 2005; Marcelino *et al*. 2020; Raven & Wagner 2021). Our study highlights the need to consider the potential niche overlap between target and sympatric species, especially when adopting conservation measures that may alter the composition of communities, such as those based on nest-site provisioning.

We would like to thank Liga para a Proteção da Natureza (LPN) for granting us access to the colonies with the Castro Verde Special Protection Area, and the Instituto da Conservação da Natureza e Florestas (ICNF) for granting us the permission to sample nests. We would also like to thank all the anonymous reviewers for the generous and constructive comments that have improved this manuscript.This work was supported by Fundação para a Ciência e a Tecnologia (FCT) and Ministério da Ciência, Tencologia e Ensiono Superior (MCTES) through grants to cE3c – Centre for Ecology, Evolution and Environmental Changes (UIBD/00329/2020), CESAM - Centre for Environmental and Marine Studies (UIDP/50017/2020 and UIDB/50017/2020), and InBIO - Research Network in Biodiversity and Evolutionary Biology (UID/BIA/50027/2013). JG and JM were supported by FCT doctoral grants (PD/BD/128366/2017 and SFRH/BD/114683/2016, respectively) and IC and TC by FCT contracts (DL57/2016/CP1440/CT0023 and IF/00694/2015, respectively).

Author contributions: JG: conceptualization, methodology, investigation, formal analysis, validation; writing – original draft preparation, review & editing; TC: conceptualization, methodology, investigation, validation; writing – review & editing; JM: investigation; writing – review & editing; AF: supervision; writing: review & editing; JP: supervision; writing – review & editing; IC: supervision, conceptualization, methodology, investigation, validation, writing – review & editing.

Availability of data: The data that support the findings of this study are available from the corresponding author upon reasonable request.

References

Alonso, H., Granadeiro, J.P., Paiva, V.H., Dias, A.S., Ramos, J.A. & Catry, P. 2012. Parent – offspring dietary segregation of Cory's Shearwaters breeding in contrasting environments. *Mar. Biol.* **159**: 1197–1207. https://doi.org/10.1007/s00227-012-1900-2.

Antón-Tello, M., Britto, V.O., Gil-Delgado, J.A., Rico, E., Dies, J.I., Monrós, J.S. & Vera, P. 2021. Unravelling diet composition and niche segregation of colonial waterbirds in a Mediterranean wetland using stable isotopes. *Ibis* **163**: 913-927. https://doi.org/10.1111/ibi.12928.

Birdlife International. 2021. IUCN Red List of Birds. http://www.birdlife.org. Accessed 07 May 2021.

Birt, V.L., Birt, T.P., Goulet, D., Cairns, D.K. & Montevecchi, W.A. 1987. Ashmole's halo: direct evidence for prey depletion by a seabird. *Mar. Ecol. Prog. Ser.* **40**: 205-208.

Casagrande, S., Nieder, L., Di Minin, E., La Fata, I. & Csermely, D. 2008. Habitat utilization and prey selection of the Kestrel *Falco tinnunculus* in relation to small mammal abundance. *Ital. J. Zool.* **75**: 401-409. https://doi.org/10.1080/11250000802085526.

Catry, I., Alcazar, R., Franco, A.M.A. & Sutherland, W. 2009. Identifying the effectiveness and constraints of conservation interventions: A case study of the endangered Lesser Kestrel. *Biol. Conserv.* **142**: 2782–2791. https://doi.org/10.1016/j.biocon.2009.07.011.

Catry, I., Franco, A.M.A., Rocha, P., Alcazar, R., Reis, S., Cordeiro, A., Ventim, R., Teodósio, J. & Moreira, F. 2013. Foraging habitat quality constrains effectiveness of artificial nest-site provisioning in reversing population declines in a colonial cavity nester. *PLoS ONE*. **8**: 1–10. https://doi.org/10.1371/journal.pone.0058320.

Catry, I., Catry, T., Alho, M., Franco, A.M.A. & Moreira, F. 2016a. Sexual and parent-offspring dietary segregation in a colonial raptor as revealed by stable isotopes. *J. Zool.* **299**: 58–67. https://doi.org/10.1111/jzo.12324.

Catry, I., Marcelino, J., Franco, A.M.A. & Moreira, F. 2017a. Landscape determinants of European Roller foraging habitat: implications for the definition of agri-environmental measures for species conservation. *Biodivers. Conserv*. **26**: 553-566. https://doi.org/10.1007/s10531-016-1241-4.

Catry, I., Sampaio, A., Silva, M.C., Moreira, F., Franco, A.M.A. & Catry, T. 2019. Combining stable isotope analysis and conventional techniques to improve knowledge of the diet of the European Roller *Coracias garrulus*. *Ibis* **161**: 272-285. https://doi.org/10.1111/ibi.12625.

Catry, T., Ramos, J., Le Corre, M., Kojadinovic, J. & Bustamante, P. 2008. The role of stable isotopes and mercury concentrations to describe seabird foraging ecology in tropical environments. *Mar. Biol.* **155**: 637-647. https://doi.org/10.1007/s00227-008-1060-6.

Catry, T. & Catry, I. 2019. Nest-site provisioning re-shapes species interactions within bird assemblages. *Ibis* **161**: 699–704. https://doi.org/10.1111/ibi.12731.

Catry, T., Lourenço, P.M., Lopes, R.J., Carneiro, C., Alves, J.A., Costas, J., Rguibi-Idrissi, H., Bearhop, S., Piersma, T. & Granadeiro, J.P. 2016b. Structure and functioning of intertidal food webs along an avian flyway: a comparative approach using stable isotopes. *Funct. Ecol.* **30**: 468-478. https://doi.org/10.1111/1365-2435.12506.

Catry, T., Moreira, F., Alcazar, R., Rocha, P.A. & Catry, I. 2017b. Mechanisms and fitness consequences of laying decisions in a migratory raptor. *Behav. Ecol.* **28**: 222-232. https://doi.org/10.1093/beheco/arw150.

Caut, S., Angulo, E. & Courchamp, F. 2009. Variation in discrimination factors (Δ^{15}N and Δ^{13}C): the effect of diet isotopic values and applications for diet reconstruction. *J. Appl. Ecol.* **46**: 443-453. https://doi.org/10.1111/j.1365-2664.2009.01620.x.

Charter, M., Izhaki, I. & Roulin, A. 2018. The relationship between intra–guild diet overlap and breeding in owls in Israel. *Popul. Ecol.* **60**: 397–403. https://doi.org/10.1007/s10144-018-0633-6.

Clewlow, H.L., Takahashi, A., Watanabe, S., Votier, S.C., Downie, R. & Ratcliffe, N. 2019. Niche partitioning of sympatric penguins by leapfrog foraging appears to be resilient to climate change. *J. Anim. Ecol.* **88**: 223-235. https://doi.org/10.1111/1365-2656.12919.

Dehnhard, N., Achurch, H., Clarke, J., Michel, L.N., Southwell, C., Sumner, M.D., Eens, M. & Emmerson, L. 2020. High inter- and intraspecific niche overlap among three sympatrically breeding, closely related seabird species: Generalist foraging as an adaptation to a highly variable environment? *J. Anim. Ecol.* **89**: 104–119. https://doi.org/10.1111/1365-2656.13078.

Duckworth, R.A., Hallinger, K.K., Hall, N. & Potticary, A.L. 2017. Switch to a novel breeding resource influences coexistence of two passerine bird. *Front. Ecol. Evol.* **5**: 1-11. https://doi.org/10.3389/fevo.2017.00072.

Forero, M.G., Bortolotti, G.R., Hobson, K.A., Donazar, J.A. & Bertelloti, M. 2004. High trophic overlap within the seabird community of Argentinean Patagonia: a multiscale approach. *J. Anim. Ecol.* **78**: 789-801. https://doi.org/10.1111/j.0021-8790.2004.00852.x.

Gameiro, J., Franco, A.M.A., Catry, T., Palmeirim, J.M. & Catry, I. 2020. Long-term persistence of conservation-reliant species: challenges and opportunities. *Biol. Conserv.* **243**: 108452. https://doi.org/10.1016/j.biocon.2020.108452.

Hardin, G. 1960. The competitive exclusion principle. *Science* **131**: 1292-1297.

Harper, M.J., McCarthey, M.A. & van der Ree, R. 2005. The use of nest boxes in urban natural vegetation remnants by vertebrate fauna. *Wildl. Res.* **32**: 509-516. https://doi.org/10.1071/WR04106.

Högstedt, G. 1980. Resource partitioning in Magpie *Pica pica* and Jackdaw *Corvus monedula* during the breeding season. *Ornis Scand.* **11**: 110–115.

Inger, R. & Bearhop, S. 2008. Applications of stable isotope analyses to avian ecology. *Ibis* **150**: 447–461. https://doi.org/10.1111/j.1474-919X.2008.00839.x.

Jackson, A. & Parnell, A. 2020. SIBER: Stable Isotope Bayesian Ellipses in R. Retrieved from https://cran.r-project.org/web/packages/SIBER/index.html.

Jaksić, F.M., Seib, R.L. & Herrera, C.M. 1982. Predation by the Barn Owl (*Tyto alba*) in Mediterranean habitats of Chile, Spain and California: a comparative approach. *Am. Midl. Nat.* **107**: 152–159. https://doi.org/10.2307/2425196.

Jenkins, E.J. & Davoren, G.K. 2020. Seabird species- and assemblage-level isotopic niche shifts associated with changing prey availability during breeding in coastal Newfoundland. *Ibis* **163**: 183-196. https://doi.org/10.1111/ibi.12873.

Kappeler, P.M., Cremer, S. & Nunn, C.L. 2015. Sociality and health: Impacts of sociality on disease susceptibility and transmission in animal and human societies. *Philos. Trans. R. Soc. B* **370**: 20140116. https://doi.org/10.1098/rstb.2014.0116

Logan, J.M., Jardine, T.D., Miller, T.J., Bunn, S.E., Cunjak, R.A. & Lutcavage, M.E. 2008. Lipid corrections in carbon and nitrogen stable isotope analyses: Comparison of chemical extraction and modelling methods. *J. Anim. Ecol.* **77**: 838–846. https://doi.org/10.1111/j.1365-2656.2008.01394.x.

MacArthur, R.H. & Pianka, E.R. 1966. On optimal use of a patchy environment. *Am. Nat.* **100**: 603-609.

Mainwaring, M.C. 2015. The use of man-made structures as nesting sites by birds: A review of the costs and benefits. *J. Nat. Conserv.* **25**: 17-22. https://doi.org/10.1016/j.jnc.2015.02.007.

Marcelino, J., Silva, J.P., Gameiro, J., Silva, A., Rego, F.C., Moreira, F. & Catry, I. 2020. Extreme events are more likely to affect the breeding success of lesser kestrels than average climate change. *Sci. Rep.* **10**: 1–11. https://doi.org/10.1038/s41598-020-64087-0.

Moreira, F., Leitão, P.J., Morgado, R., Alcazar, R., Cardoso, A., Carrapato, C., Delgado, A., Geraldes, P., Gordinho, L., Henriques, I., Lecoq, M., Leitão, D., Marques, A.T., Pedroso, R., Prego, I., Reino, L., Rocha, P., Tomé, R. & Osborne, P.E. 2007. Spatial distribution patterns, habitat correlates and population estimates of steppe birds in Castro Verde. *Airo* **17**: 5-30.

Motis, A., Estrada, J. & Oro, D. 1997. Nestling diet of the Spotless starling *Sturnus unicolor* and the European Starling *Sturnus vulgaris* in a sympatric breeding area. *Ornis Fenn.* **74**: 179–185.

Murton, R.K. & Westwood, N.J. 1966. The foods of the rock dove and feral pigeon. *Bird Study* **13**: 130–146. https://doi.org/10.1080/00063656609476116.

Olah, G., Vigo, G., Heisohn, R. & Brightsmith, D.K. 2014. Nest site selection and efficacy of artificial nests for breeding success of Scarlet Macaws *Ara macao macao* in lowland Peru. *J. Nat. Conserv.* **22**: 176-185. https://doi.org/10.1016/j.jnc.2013.11.003.

Orihuela-Torres, A., Perales, P., Rosado, D. & Pérez-García, J.M. 2017. Feeding ecology of the Common Kestrel *Falco tinnunculus* in the south of Alicante (SE Spain). *Rev. Cat. Ornitol.* **33**: 10–16.

Orłowski, G., Karg, J., Rłowski, G.O., Usiecki, S.R. & Karg, J. 2014. Partial dietary segregation between adult and nestling Bluethroats *Luscinia svecica*. *Acta Ornithol.* **49**:107–118. https://doi.org/10.3161/000164514X682931.

Pianka, E.R. 1981. Competition and niche theory. In RM May (Ed.) *Theoretical ecology* (pp. 167–196). Oxford: Blackwell.

R Core Team 2016. A Language and environment for statistical computing. Retrieved from Vienna: R Foundation for statistical computing website: https://cran.rproject.org/

Razgour, O., Korine, C. & Saltz, D. 2011. Does interspecific competition drive patterns of habitat use in desert bat communities? *Oecologia* **167**: 493-502. https://doi.org/10.1007/s00442-011-1995-z.

Raven, P.H. & Wagner, D.L. 2021. Agricultural intensification and climate change are rapidly decreasing insect biodiversity. *PNAS* **118**: 1-6. https://doi.org/10.1073/pnas.2002548117.

Reisinger, R.R., Carpenter-Kling, T., Connan, M., Cherel, Y. & Pistorius, P.A. 2020. Foraging behaviour and habitat-use drives niche segregation in sibling seabird species: Niche segregation in giant petrels. *R Soc. Open. Sci.* **7**: 200649 . https://doi.org/10.1098/rsos.200649.

Riegert, J., Šindelář, J., Zárybnická, M. & Horáček, I. 2021. Large-scale spatial patterns of small-mammal communities in the Mediterranean region revealed by Barn owl diet. **Sci. Rep. 11**: 4985. https://doi.org/10.1038/s41598-021-84683-y.

Šalek, M. & Lövy, M. 2012. Spatial ecology and habitat selection of Little Owl *Athene noctua* during the breeding season in central European farmland. *Bird Conserv. Int.* **22**: 328-338. https://doi.org/10.1017/S0959270911000268.

Schuette, P., Wagner, A.P., Wagner, M.E. & Creel, S. 2013. Occupancy patterns and niche partitioning within a diverse carnivore community exposed to anthropogenic pressures. *Biol. Conserv.* **158**: 301-312. https://doi.org/10.1016/j.biocon.2012.08.008.

Silva, J.P., Correira, R., Alonso, H., Martins, R.C., D'Amico, M., Delgado, A., Sampaio, H., Godinho, C. & Moreira, F. 2018. EU protected area network did not prevent a country wide population decline in a threatened grassland bird. *Peer J* **6**: 1-13. https://doi.org/10.7717/peerj.4284.

Soler, M., Alcata, N. & Soler, J.J. 1990. Alimentación de la Grahilla *Corvus monedula* en tres zonas del sur de España. *Doñana Acta Vertebrata* **17**, 17-48.

Sutherland, W.J., Dicks, L.V., Ockendon, N., Petrovan, S.O. & Smith, R.K. 2018. *What works in conservation*. Cambridge: Open Book Publishers.

Teckentrup, L., Kramer-Schadt, S. & Jeltsch, F. 2019. The risk of ignoring fear: underestimating the effects of habitat loss and fragmentation on biodiversity. *Landsc. Ecol.* **34**: 2851-2868. https://doi.org/10.1007/s10980-019-00922-8.

Tomé, R., Catry, P., Bloise, C. & Korpimäki, E. 2008. Breeding density and success, and diet composition of Little Owls *Athene noctua* in steppe-like habitats in Portugal. *Ornis Fenn.* **85**: 22-32.

Tscharntke, T., Klein, A.M., Kruess, A., Steffan-Dewenter, I. & Thies, C. 2005. Landscape perspectives on agricultural intensification and biodiversity – ecosystem service management. *Ecol. Lett.* **8**: 857-874. https://doi.org/10.1111/j.1461-0248.2005.00782.x.

Turner, T.F., Collyer, M.L. & Krabbenhoft, T.J. 2010. A general hypothesis-testing framework for stable isotope ratios in ecological studies. *Ecology* **91**: 2227-2233. https://doi.org/10.1890/09-1454.1.

Wiegand, T., Revilla, E., Moloney, K.A. 2005. Effects of habitat loss and fragmentation on population dynamics. *Conserv. Biol.* **19**: 108-121. https://doi.org/10.1111/j.1523-1739.2005.00208.x.

Weiss, F., Furness, R.W., McGill, R.A.R., Strange, I.J., Masello, J.F. & Quillfeldt, P. 2009. Trophic segregation of Falkland Islands seabirds: Insights from stable isotope analysis. *Polar Biol.* **32**: 1753–1763. https://doi.org/10.1007/s00300-009-0674-6.

Tables

Table 1: *Carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) stable isotope signatures (mean ± SD) of six bird species breeding in mixed colonies in Castro Verde, Southern Portugal. Nestling feathers and blood (plasma) from parent-offspring pairs were collected during the breeding seasons of 2014-2018 (pooled) and 2019, respectively. n = sample size.*

	Feathers (nestlings)			**Blood**			
	δ^{13}C	δ^{15}N	n		δ^{13}C	δ^{15}N	n
Western Barn Owl	-23.42 ± 0.58	10.64 ± 0.45	12	-	-	-	-
Little Owl	-23.81 ± 1.07	10.08 ± 1.37	19	Nestling	-25.21 ± 0.45	10.03 ± 1.11	7
				Adult	-25.63 ± 0.34	11.15 ± 0.74	7
Common Kestrel	-23.64 ± 0.55	8.88 ± 1.05	33	Nestling	-25.28 ± 0.34	8.77 ± 0.63	8
				Adult	-25.20 ± 0.44	10.07 ± 0.47	8
Lesser Kestrel	-23.78 ± 0.25	7.76 ± 0.66	36	Nestling	-25.55 ± 0.19	7.93 ± 0.76	11
				Adult	-25.38 ± 0.40	8.94 ± 0.91	11
European Roller	-23.52 ± 0.42	7.34 ± 0.69	36	Nestling	-25.42 ± 0.26	7.30 ± 0.67	6

				Adult	-25.17 ± 0.23	8.78 ± 1.14	6
Spotless Starling	-23.84 ± 0.52	8.54 ± 0.89	12	Nestling	-25.48 ± 0.26	6.62 ± 1.03	6
				Adult	-25.49 ± 0.20	8.61 ± 1.02	6

Table 2: *Carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) stable isotope signatures (mean ± SD) of main prey sampled in Castro Verde, Southern Portugal. Taxa within coleoptera and orthoptera were grouped into SIA groups according to their similarity in isotopic signatures. n = sample size.*

	$\delta^{13}C$	$\delta^{15}N$	n	SIA group
Small mammals				
Apodemus sp.	-25.6 ± 0.8	7.0 ± 1.9	7	
Mus sp.	-25.3 ± 0.4	8.1 ± 0.9	3	
Soricidae	-24.9 ± 0.3	8.2 ± 1.6	2	
Birds				
Coturnix sp.	-26.1 ± 0.3	7.2 ± 0.5	2	
Sturnus unicolor	-25.3 ± 0.6	8.5 ± 1.2	3	
Reptiles				
Chalcides striatus	-25.3 ± 0.5	8.2 ± 0.8	8	
Chilopoda				
Scolopendra sp.	-25.9 ± 0.8	9.0 ± 0.7	8	
Coleoptera				
Carabidae und.	-25.5 ± 0.5	10.9 ± 3.0	3	1
Silpha sp.	-25.5 ± 0.7	10.8 ± 3.4	3	1
Tenebrionide und.	-26.3 ± 0.3	9.7 ± 0.2	2	1
Amphimllon nigrum	-26.6 ± 0.8	6.6 ± 0.9	10	2
Carabus rugosus	-26.7 ± 0.8	5.9 ± 1.0	2	2
Chrysolina bankii	-27.1 ± 0.2	7.0 ± 1.0	3	2
Pterostichus ebenus	-25.9 ± 0.1	7.0 ± 0.2	2	2
Sepidium sp.	-26.8 ± 0.8	6.5 ± 0.7	8	2
Bubas bison	-27.3 ± 0.6	8.9 ± 0.4	5	3
Netocia sp.	-23.2 ± 0.3	5.6 ± 3.7	2	4
Protaetia sp.	-23.9	7.4	1	4
Orthoptera				
Acrididae	-26.4 ± 0.7	3.8 ± 1.0	12	5
Tettigonidae	-25.3 ± 0.7	3.4 ± 1.5	14	5
Gryllotalpa sp.	-27.2 ± 0.7	6.4 ± 1.0	7	6

Table 3: *Isotopic niche breadth of nestlings (feathers) and adults (plasma) from bird species breeding in mixed colonies. Bayesian standard ellipse areas (SEAb, with 95% credible intervals) and sample size-corrected standard ellipse areas (SEAc) are shown. n = sample size.*

Consumers	**n**	**SEA_B (95% credible interval)**	**SEA_C**
Nestlings (feathers)			
Western Barn Owl	12	0.755 (0.439-1.451)	0.899
Little Owl	19	4.352 (2.824-7.175)	4.848
Common Kestrel	33	1.763 (1.265-2.543)	1.872
Lesser Kestrel	36	0.505 (0.369-0.718)	0.535
European Roller	36	0.830 (0.602-1.173)	0.871
Spotless Starling	12	1.320 (0.770-2.540)	1.575
Adults (plasma)			
Little Owl	7	0.688 (0.333-1.682)	0.922
Common Kestrel	8	0.558 (0.290-1.297)	0.736
Lesser Kestrel	11	1.058 (0.590-2.064)	1.254
European Roller	6	0.578 (0.274-1.592)	0.785
Spotless Starling	6	0.532 (0.248-1.446)	0.764

Figure legends

Commented [RF1]: update legend to full species names, and capitalise each word

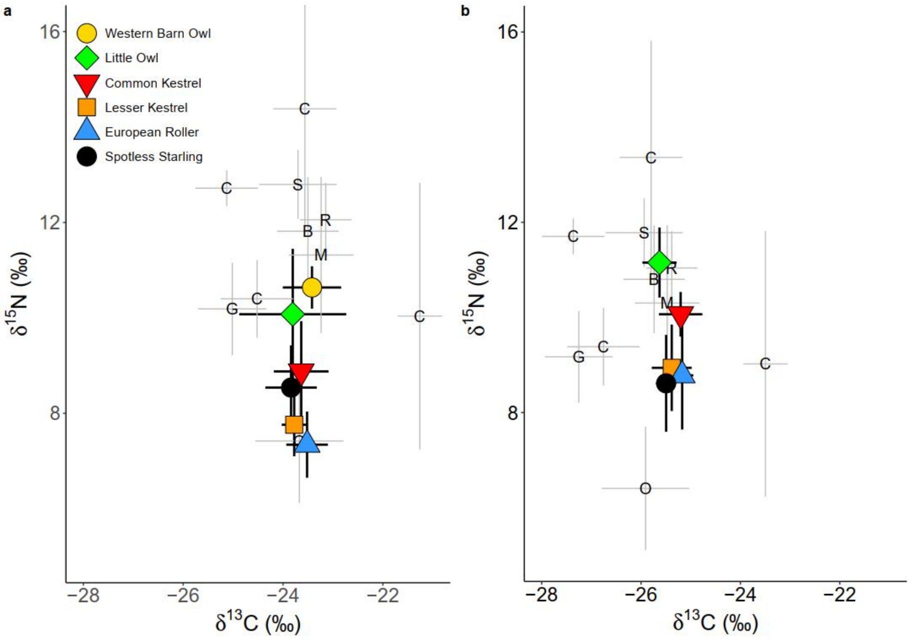

Fig. 1: *Carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) stable isotope signatures (mean ± SD) of nestlings (A) and adults (B) from six bird species breeding in mixed colonies established in an artificial nesting landscape in Southern Portugal. Isotope signatures of nestlings and adults were obtained from feathers (collected in 2014, 2016, and 2018, pooled) and blood plasma (collected in 2019), respectively. Signatures of main prey items are shown. M, small mammals; B, birds; R, reptiles; S, scolopendra; C, coleoptera; O, orthoptera; G, Gryllotalpa. Stable isotope ratios of prey items were obtained from muscle/soft tissue and corrected for the trophic discrimination factor (based on Caut et al. 2009, see methods). Because different tissues were used for nestlings and adults, direct comparisons in trophic position between the two age groups should be avoided.*

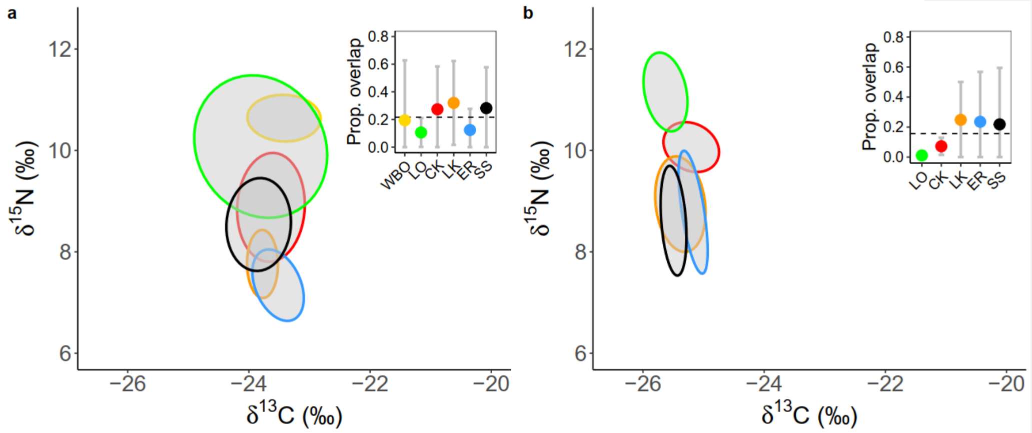

Fig. 2: *Overlap in isotopic niches (corrected standard ellipses; SEA$_C$) among nestlings (A) and adults (B) from six bird species breeding in mixed colonies established in an artificial breeding landscape in Southern Portugal. WBO = Western Barn Owl, LO = Little Owl, CK = Common Kestrel, LK = Lesser Kestrel, ER = European Roller, SS = Spotless Starling. Isotope signatures of nestlings and adults were obtained from feathers and blood plasma, respectively. Inset plot (upper right corner) shows the mean (±SD) niche overlap for each species and the mean overlap of the community (horizontal dashed line).*

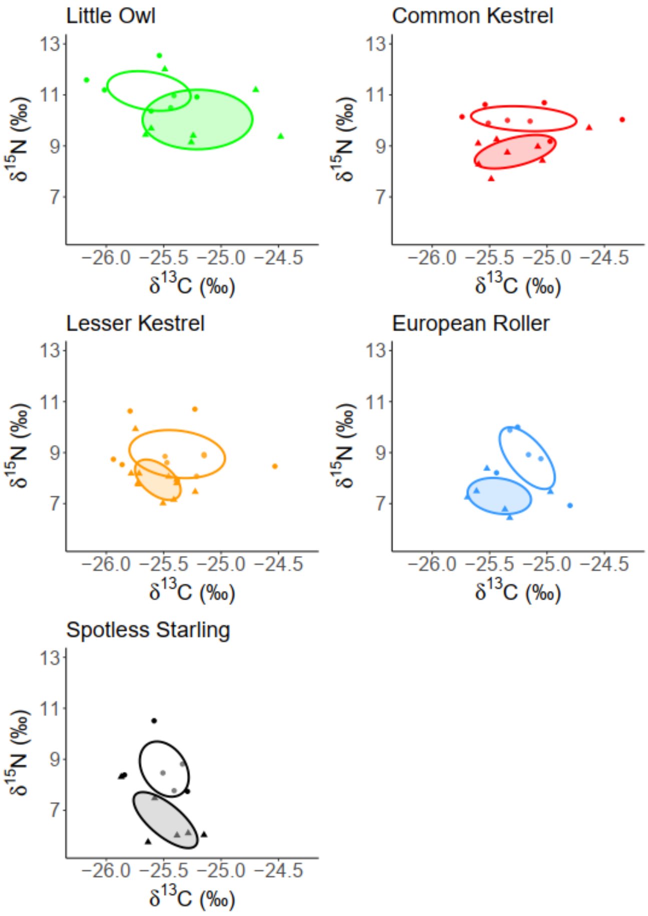

Fig. 3 *Overlap in isotopic niches (corrected standard ellipses; SEAc) between parents (clear) and their offspring (filled) of bird species breeding in mixed colonies established in an artificial nesting landscape in Southern Portugal. Stable isotope ratios ($\delta^{13}C$ and $\delta^{15}N$) were measured using blood plasma from 7 Little Owl, 8 Common Kestrel, 11 Lesser Kestrel, 6 Roller, and 6 Starling parent-offspring pairs (one parent and one offspring in each pair). Circles and triangles show individual signatures of parents and nestlings, respectively.*