High trophic niche overlap in mixed bird colonies relying on artificial nests

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ABSTRACT: 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 several bird species forming mixed breeding assemblages, likely promoting 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 Kestrel *Falco tinnunculus*, European Roller *Coracias garrulus*, Barn Owl *Tyto alba*, Little Owl *Athene noctua*, and Spotless Starling *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 guarantee 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 need to consider the ecological niches of target and sympatric species as well as their interactions.

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

1. Introduction

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 and massive provisioning of artificial nest-sites to halt species decline across 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 are often overlooked by researchers or conservation managers (Mainwaring et al. 2015). According to the 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). In the event this new and artificial nesting landscape promotes the aggregation of potential competitor species, artificial nest-sites may turn into ecological trps by reducing long-term individual fitness associated with 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 (like in insectivorous desert bats, Razgour et al. 2011), different periods of the day (like in African carnivore communities, Schuette et al. 2013), or when they have different breeding phenologies (like in sympatric penguin species, Clewlow et al. 2019). Segregation in the dietary axis occurs when species reduce their dietary niche overlap by foraging for alternative food resources (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 dietary sexual or parent-offspring segregation, especially when the potential for competitive interactions is maximum, 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-specific bird community 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 low nesting opportunities, many bird species took advantage of these artificial breeding structures – some with more than 80 cavities, forming mixed-species colonies (Catry & Catry 2019). These include Lesser Kestrels, Common Kestrels *Falco tinnunculus*, European Rollers *Coracias garrulus*, Barn Owls *Tyto alba*, Little Owls *Athene noctua*, Jackdaws *Corvus monedula*, Spotless Starling *Sturnus unicolor*, and Feral Pigeons *Columba livia* (Catry & Catry 2019). The dietary habits of most of these species – large arthropods to small mammals – 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 different species and (2) assess parent-offspring diet segregation of each species within the assemblages. We sampled feathers from nestlings and blood plasma from parent-offspring pairs during the breeding season, thus providing a detailed spectrum of 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. Altogether, we aimed at understanding the extent on how dietary overlap and niche segregation among potential competitors may affect the conservation of targeted species, an issue seldom acknowledged by conservation projects.

2. Methods:

2.1 Study area and study species

This study was carried out in the Castro Verde Special Protection Area (SPA, Natura 2000), Portugal (37°41'N, 8°05'W). With ca. 85,000 ha, this is the main Portuguese area of agro-steppes – 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 the last 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). 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. Within these breeding assemblages, we focused on species that prey mainly on small vertebrates and arthropods, as these are the ones with higher potential for having overlapped trophic niches: Barn and Little Owls, Common and Lesser Kestrels, European Rollers (hereafter Rollers) and Spotless Starlings (hereafter Starlings). Feral Pigeons and Jackdaws, due to the relatively large proportion of plant sources in their diets (cereals; Murton & Westwood 1966; Högstedt 1980; Soler et al. 1990), were not included in the study.

2.2. Sample collection

Feather sampling was carried out during the breeding seasons of 2014, 2016 and 2018 (Table 1). Nestlings of Barn Owls (n=12), Little Owls (n=19), Common Kestrels (n=33), Lesser Kestrels (n=36), Rollers (n=36), and 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 (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), Rollers (n=6), and Starlings (n=6) was carried out during the breeding season of 2019 (Table 1). Parents and their offspring were sampled within a close 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 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 breeding season of 2018 (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).

2.3. Stable isotope analysis

Stable isotope ratios have been widely used as dietary tracers to assess the different habitats and diet resources used by consumers, providing a proxy for species or individual ecological niches using low invasive methods (Forero et al. 2004; Catry et al. 2019; Dehnhard et al. 2020). SIA 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 the dietary niche segregation/overlap of multiple species or individuals within assemblages and infer on their competition for resources (Inger & Bearhop 2008; Alonso et al. 2012; Catry et al. 2016b; Reisinger et al. 2020; Jenkins and Davoren, 2020). SIA 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 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 (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 much higher turnover rate, reflecting the averaged diet over much shorter periods (days), and thus allows for a proper comparison between parent and offspring signatures (days; 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 reduced to a homogenised 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 3400rev/min, and the supernatant containing solvent and lipids were 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 to 1.2mg 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 ration 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).

To search for differences in isotopic signatures among species, pairwise multivariate analyses of variance were 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 analysis were 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 evaluating for data normality. 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 discrimination 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 standard ellipses (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 R statistical environment (R Development Core Team, 2016).

3. Results

3.1 Stable isotope signatures

Feathers were collected from a total of 148 nestlings and plasma samples from 38 parent-offspring pairs from 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 Rollers ($p = 0.02$). 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 Rollers, (both $p < 0.001$), but similar values to Starlings ($p = 0.257$). Starlings also had more enriched $\delta^{15}N$ than Lesser Kestrels ($p = 0.016$) and Rollers ($p < 0.001$) and Lesser Kestrels had higher values than 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 Barn Owls were not sampled), with Little Owls being in a significantly higher position than Lesser Kestrels, Rollers, and Starlings (all $p < 0.001$), while Common Kestrels had only significantly higher $\delta^{15}N$ values than Starlings (p-values: Lesser Kestrel = 0.061, Roller = 0.068, Starling = 0.030). Adult Lesser Kestrels, Rollers and 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 Barn Owl = Little Owl > Common Kestrel = Starling > Lesser Kestrel > Roller; and Little Owl = Common Kestrel > Starling = Lesser Kestrel = Roller; respectively.

Isotopic signature of main prey items (corrected by TEF) can be found on 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, Rollers, and Starlings feed their offspring mostly with Orthoptera and to a lesser extent with Coleoptera and vertebrates, while the opposite occurs in Barn and Little Owls (Fig. 1A). Regarding adults, Little Owls and Common Kestrels had closer signatures to vertebrates, while Lesser Kestrels, Rollers, and 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., Barn Owls and Little Owls, and those preying mostly on arthropods, such as Lesser Kestrels, Rollers, and 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 Starlings (23% and 12%, respectively; Table S1) but totally overlapping with Barn Owls ($SEA_B = 0.755$; Table 3) (Fig. 2A). Common Kestrels and 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 Rollers, Common Kestrels, and Starlings (60%, 40% and 60%, respectively; Table S1). Amongst adults, Lesser Kestrels, Rollers, and 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 $\delta^{15}N$ 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 $w_{(20)} = 107$, $p = 0.001$; Roller: $t_{(10)} = 2.753$, $p = 0.025$; Starling: $w_{(10)} = 34$, $p = 0.009$). No differences in $\delta^{13}C$ 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: $t_{(20)} = 1.256$, $p = 0.228$; Roller: $t_{(10)} = 0.115$, $p = 0.115$; 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 clear 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 draw adequate conservation management actions. 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 δ15N isotopic signatures of Lesser Kestrel and 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 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 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 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. Regarding Starlings, although there is no information on their diet in our study area, in general they rely mostly on small insects such as hoverflies, ants, and on larvae of Coleoptera and Lepidoptera (Motis et al. 1997), which should give Starlings a distinct isotope signature. However, in our study area Starlings were often observed feeding their offspring with grasshoppers, suggesting a similar diet and 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 Rollers showed a high overlap with Starlings, suggesting similar diets, while Common Kestrels and Little Wwls 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 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 exhibited marked parent-offspring segregation in trophic resources, reinforcing 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 the 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 we studied, there is no evidence for temporal or spatial segregation, as all species highly coincide in their breeding phenology and chick rearing period, and all hunt in the vicinity of the colonies as central-place foragers (Casagrande et al. 2008; Catry et al. 2012; Šalek & Lövy 2012; Catry et al. 2017a). Different species could still select for different (micro) habitats or hunting strategies that would foster niche segregation, an aspect that should be investigated in future studies.

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 Rollers (and likely of the whole species assemblage except 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 prey that became temporarily super-abundant, increasing their overlap without necessarily increasing interspecific competition (Forero et al. 2004; Jenkins & Davoren 2020). The lack of differences in productivity between 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.

4.4 Conclusion

Conservation actions based on nest provisioning are often successful at quickly recover endangered populations, but seldom consider how they may shape the composition of communities and alter interspecific interactions (Catry & Catry 2019). In the sequence of the implementation of European Union LIFE European projects focused on the recovery of Lesser Kestrels in Portugal, the provisioning of more than a thousand artificial nest-sites attracted many other avian species. Interference competition and even predation have already been reported among most species breeding in these colonies (Catry & Catry 2019), and the high trophic overlap reported in this study may be another trigger responsible for the observed interspecific antagonistic behaviours. This may particularly affect Lesser Kestrels and Rollers, two species of conservation concern, classified as Vulnerable and Critically endangered in Portugal, respectively, and with decreasing population trends throughout their breeding ranges (Cabral et al. 2005; BirdLife International 2021). Food resources are abundant in the study area, so interspecific competition for food may not be a problem here. It remains unknown whether the high overlap reported here would still occur in areas with limited resource availability or if species would decrease their trophic niche overlap to avoid competition (Pianka 1981). Future studies should address how sympatric species coexist under these scenarios, which are predicted to increase with ongoing human and climate-induced habitat changes (Tcharntke et al. 2005; Marcelino et al. 2020; Raven & Wagner 2021). Conservation practices based on nest-site provisioning should nonetheless consider the ecological niche of both target and sympatric species, and ensure suitable foraging habitat with high resource availability in order to secure the long-term viability of threatened species in these new, human-shaped nesting landscapes."

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

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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

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
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
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**	**$\mathrm{SEA}_{\mathrm{B}}$ (95% credible interval)**	**$\mathrm{SEA}_{\mathrm{C}}$**
Nestlings (feathers)			
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
Roller	36	0.830 (0.602-1.173)	0.871
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
Roller	6	0.578 (0.274-1.592)	0.785
Starling	6	0.532 (0.248-1.446)	0.764

Figure legends

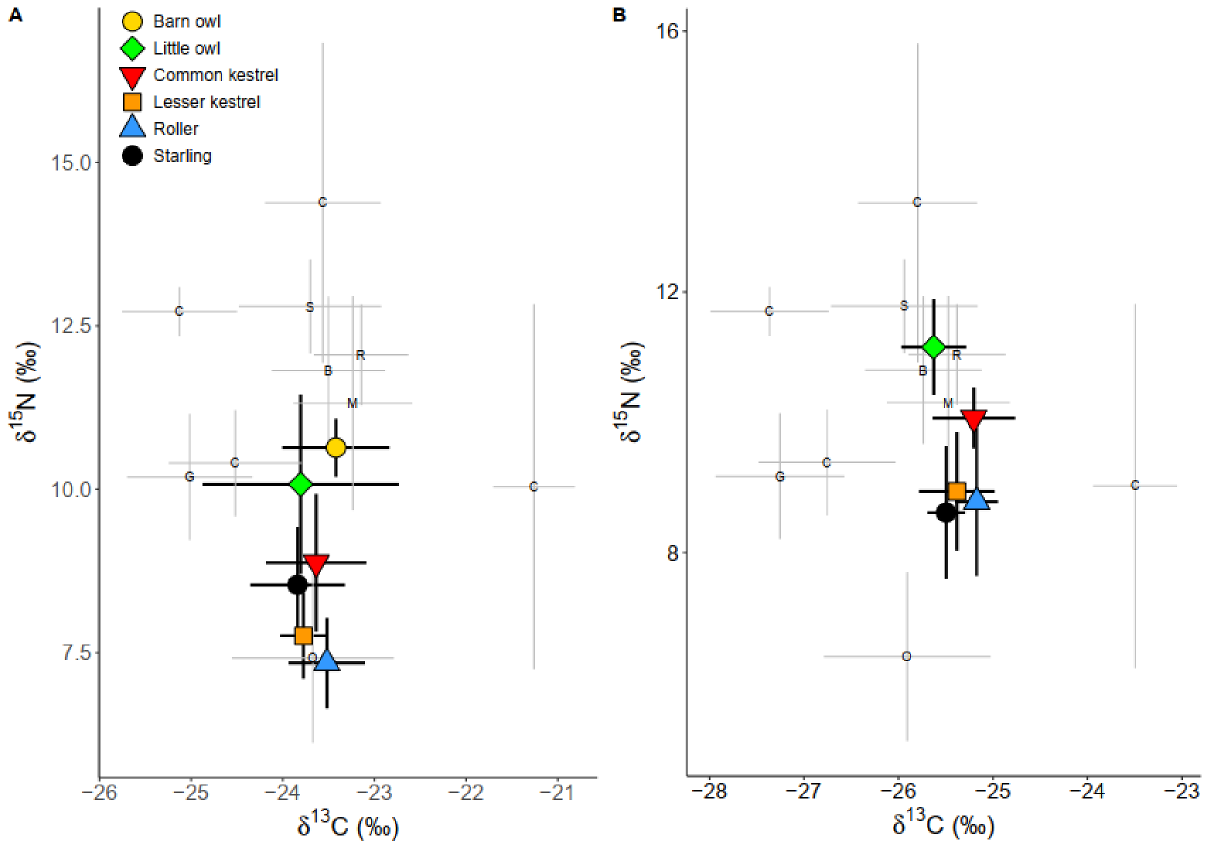

Fig. 1: *Carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) stable isotope signatures (mean ± SD) of nestlings (A) and adults (B) from six bird species breeding in mixed colonies established in an artificial nesting landscape in Southern Portugal. Isotope signatures of nestlings and adults were obtained 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.*

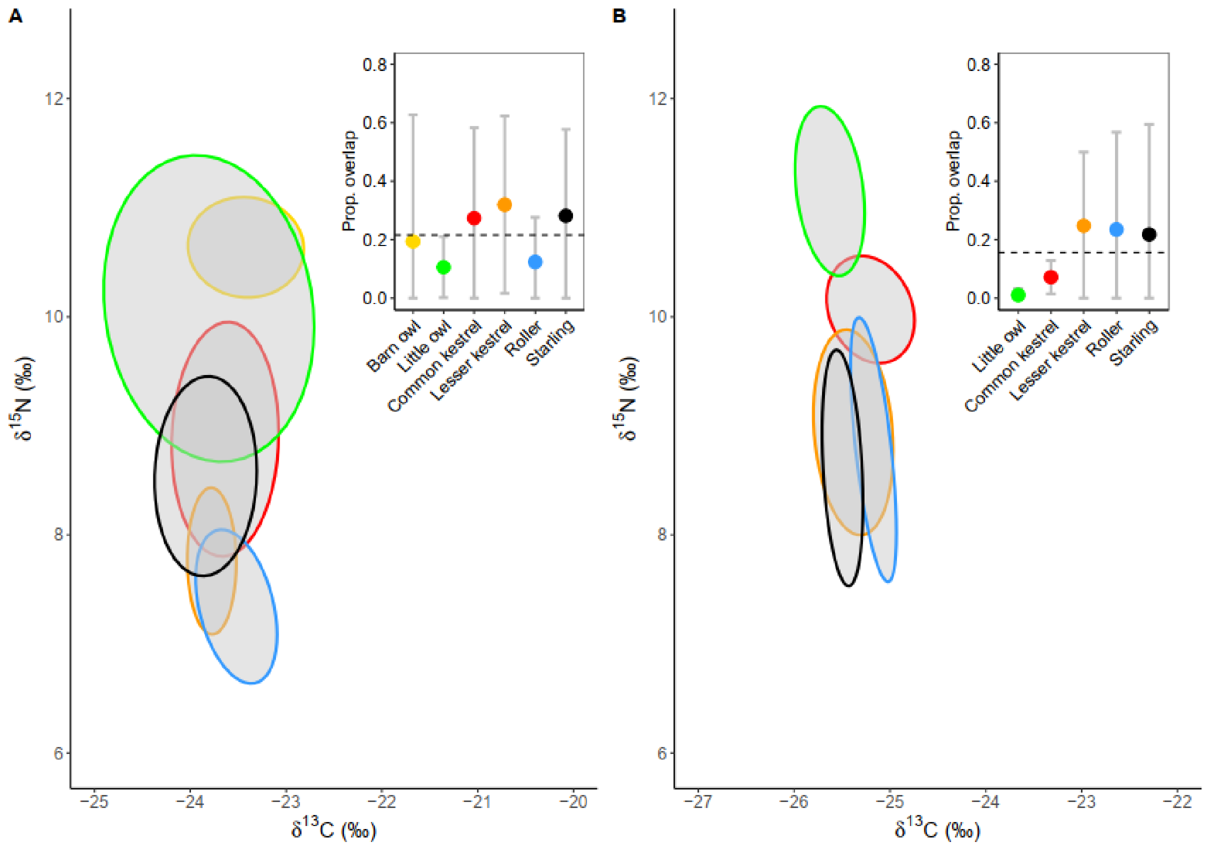

***Fig. 2:** Overlap in isotopic niches (corrected standard ellipses; SEA_C) among nestlings (A) and adults (B) from 6 bird species breeding in mixed colonies established in an artificial breeding landscape in Southern Portugal. Isotope signatures of nestlings and adults were obtained from feathers and 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).*

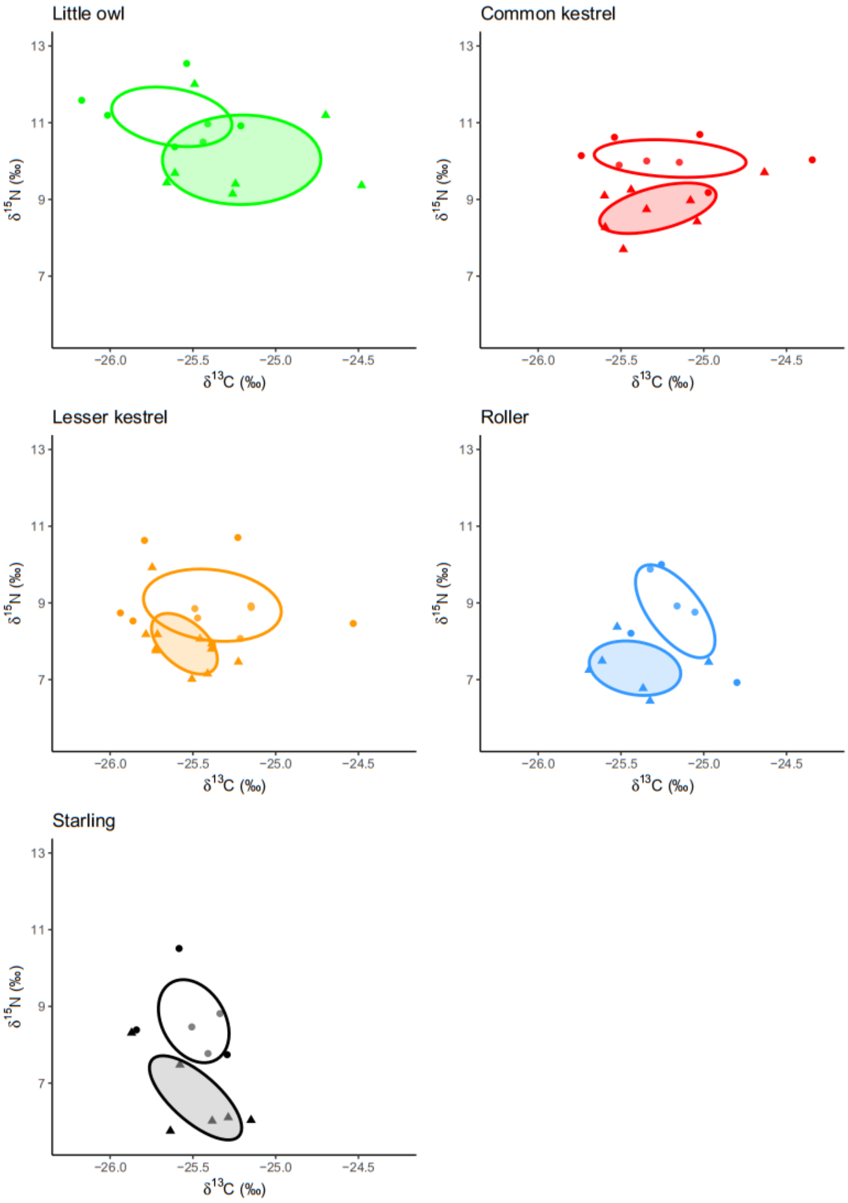

Fig. 3 *Overlap in isotopic niches (corrected standard ellipses; SEAc) between parents (clear) and their offspring (filled) of bird species breeding in mixed colonies established in an artificial nesting landscape in Southern Portugal. Stable isotope ratios ($\delta^{13}C$ and $\delta^{15}N$) were measured 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.*