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Effects of Habitat Structure and Feeding Habits on Productivity and Nestling Quality of Barn Owl *Tyto alba* (Scopoli, 1769) (Strigiformes: Tytonidae) in the Iberian Peninsula

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Abstract: European populations of the barn owl *Tyto alba* are undergoing a sharp decline. The assessment of habitat structure, feeding habits and nestling quality is relevant to the conservation of this bird of prey, particularly in the Iberian Peninsula. The aim of this study was to analyse habitat features of nesting sites and diet of chicks in order to assess their effects on brood size, body condition and nutritional status of Iberian barn owl offspring. Nests were sampled in Madrid County (central Spain) during spring 2019. After pellet analysis from chicks, prey richness and prey diversity were higher in more heterogeneous habitats (identified by data processing with GIS). Large brood sizes were more frequent next to urban areas, with greater consumption of human-associated Muridae (house mouse *Mus musculus* and brown rat *Rattus norvegicus*). Conversely, chicks showed better body condition (body mass controlled by size measurements) and nutritional status (blood samples: glucose, proteins, triglycerides and alkaline phosphatase levels) in mountainous habitats with well-developed vegetation. In this habitat type, diet was based on wild micromammals (Etruscan shrew *Suncus etruscus*, Cabrera's vole *Microtus cabrerae* and wood mouse *Apodemus sylvaticus*). Ecological, morphological and physiological traits are different indicators that provide more accurate insights into the productivity and nestling quality in the barn owl. This information may be applied by policy-makers and environmental managers for the conservation of Iberian barn owl populations.

Key words: bird of prey, blood parameters, body mass, brood size, diet

Introduction

The barn owl *Tyto alba* (Scopoli, 1769) is a common and widespread species among the owls of Europe (Bunn et al. 2010, Poprach 2010). This medium-sized nocturnal bird of prey (taxonomic Family Tytonidae, Order Strigiformes) inhabits a wide variety of habitats, from mountains (600–1200 m a.s.l.) to lowlands (<100 m a.s.l.), where it feeds mainly on micromammals; although insects, lizards, small birds and bats are also hunted (Taylor 1994, Szép et al. 2021). Moreover, this species is very tolerant to human presence and is frequently associated with anthropic environments (Salvati et al. 2002, Roulin

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2020). However, in spite of its opportunistic behaviour and generalist requirements of food and habitat, barn owl populations have undergone a sharp decline throughout Europe over the last 50 years, in some cases around 70% (Toms et al. 2001). The main reasons suggested for this decline are changes in agricultural practices, favoured by the reform of the Common Agricultural Policy (CAP), which are leading to the loss of suitable foraging habitat and nesting sites (e.g. Stoate et al. 2009). Also, the rate of habitat alteration has increased steeply over recent decades in relation to the rapid urbanization of key areas, extended road networks and an analogous increase in road traffic (Martínez & López 1995, Toms et al. 2001). The negative trend in population size has also been observed in southern Europe, particularly in the Iberian Peninsula, where barn owls are sensitive to habitat changes at a small scale (Martínez & López 1995, Martínez & Zuberogoitia 2004).

Structural habitat features, such as vegetation composition and distribution, determine variability in food resources and refuge for barn owls (Taylor 1994, Poprach 2010, Roulin 2020). Both habitat structure and prey abundance reflect territory quality and are therefore considered the main factors influencing the barn owl's decision to breed (Salvati et al. 2002, Bond et al. 2004). To assess territory quality for breeding birds, productivity and nestling quality are typically estimated using clutch and brood sizes or body size/mass of chicks (e.g. Martínez & López 1995, Deshler & Murphy 2012). Moreover, blood parameters have been shown as good indicators of nutritional status in a wide variety of species under conservation concern, from fish (Almeida & Grossman 2014) to birds of prey (Dawson & Bortolotti 1997), including nestlings (Ferrer 1994, Dobado-Berrios & Ferrer 1997). In particular, nutritional status of chicks would reflect both prey availability and food quality in a given area. Thus, blood parameters of barn owl nestlings could also contribute to the estimation of the territory quality for this species. However to date, no data on blood chemistry have been published for wild Iberian barn owls.

The assessment of environmental features (e.g. structural habitat heterogeneity) and feeding habits, particularly at nesting sites, along with their effects on the offspring are crucial to the conservation of barn owls (Bond et al. 2004, Martínez & Zuberogoitia 2004). The overall conclusions from these studies could also be highly relevant for providing accurate predictions on population trends for this species, as well as identifying areas where optimal reproductive rates can be achieved. This information may contribute to the design of conservation and recovery plans suitable for particular zones in the Iberian barn owl distribution. In temperate regions, studies analysing environmental features are used to provide advice on where to invest efforts in habitat protection and thus to reinforce population growth of barn owls (Bond et al. 2004). This would be of particular interest in the Iberian Peninsula, where information on breeding ecology of the barn owl is scarce and it is not up to date (e.g. see one of the few examples in Martínez & López 1999).

The aim of this study was to analyse productivity and nestling quality in Iberian barn owls relative to habitat structure of nesting sites and feeding habits of chicks. Specifically, the relationships between habitat features (e.g. vegetation, land use) and diet were studied in order to assess their effects on brood size, body condition and nutritional status of chicks. The hypotheses to be tested were that: 1) the diet of chicks will be more diverse in structurally more heterogeneous nesting habitats; 2) the barn owl will take advantage of urban-influenced habitats to produce larger brood sizes, where the chicks will be fed with human-associated species (Salvati et al. 2002); and 3) nestling quality, measured as body condition and nutritional status of chicks, will be better in habitats where the diet is more diverse and based on wild prey (Clum et al. 1997, Teta et al. 2012).

Materials and Methods

Study area

In Spain, the barn owl is included in the 'List of Species for Special Protection' and consequently, it is protected by specific legislation acts, i.e. 42/2007 and 139/2011 RD (Ministry of Environment – Spain 2021a). Moreover, nests are commonly found in areas of particular protection in Spain (e.g. Natural and National Parks) (Martínez & López 1999, Guerra et al. 2014) and Europe (e.g. SPAs for Birds, IBAs, The Habitats Directive) (Bond et al. 2004, Szép et al. 2021). For these reasons and for a better administrative control, field sampling was only allowed in protected areas in Spain, with a maximum of $n = 20$ nests to be sampled. Because of this low number of nests, a comprehensive regional survey was undertaken within the administrative County of Madrid (central Spain). Geographic coordinates for the Latitude and Longitude limits were: (39º50'–41º13'N and 2º58'–4º50'W, respectively). In terms of sampling design, Madrid County was selected because it is a relatively small territory (≈8000 km^2), which allows the limited sampling size (n = 20 nests) to be statistically representative of the study area. Furthermore, the region' climatic conditions are very homogeneous (Ministry of Environment – Spain 2021b), and it possesses a wide variety of habitats commonly used by the barn owl (e.g. forests, farmlands and suburban environments). According to Spanish legislation, conservation measures to preserve biodiversity must be implemented by each particular region. This was another key factor to select only one small county, as it avoids potential 'administrative bias' in the data-set.

Substratum in the study area is mainly siliceous, with some examples of gypsum and clay soils. The climate in central Spain is continental Mediterranean, with rainfall concentrated in autumn and winter (800–1000 mm), and intense summer drought (<500 mm). The average annual temperature ranges between 10–15ºC. The lowest temperatures occur in winter (−5ºC) and the highest in summer (45ºC). Vegetation varies from forests of oak (*Quercus* spp.) and pine (*Pinus* spp.) to cereal crops.

Although this was a single-year study, 2019 was a hydrologically average year for the study area, in terms of rainfall and temperatures recorded (Ministry of Environment – Spain 2021b), as well as similarly average values for ecosystem productivity and rodent-prey abundances (Ministry of Environment – Spain 2021c). These conditions increase the potential generality of the present findings and conclusions.

Field sampling

The survey to locate barn owl nests started in March 2019, as the laying date of the first clutches in this species can occur within this month (Martínez & LÓPEZ 1999). This ensured that all assessed chicks belonged to the first clutch of the year, controlling for the possible effect of successive clutches. The nests were located according to the published methodology in Zuberogoitia & Campos (1998), with all the nests being discovered during the first incubation period in the study area (i.e. from late March to May). Good accessibility to the brood was a particular criterion for nest selection, as this facilitated the manipulation of chicks and consequently, minimised the disturbance effect on juvenile development. For a better data representativeness of the study area, nesting sites were evenly distributed within Madrid County and separated by >10 km, which also minimised data dependence among sampling sites. After the nests were located, they were visited regularly to record the approximate hatching date (from late April to late May) and then to monitor the chick development until they reached the particular body mass to take blood samples and morphometric data (see below). This monitoring was also used for recording how many nestlings eventually fledged. The visits to the nests were undertaken carefully to minimise disturbance of both the parents and the chicks, reducing the consequent risk of nest abandonment. Nests were selected to be sampled only if they had been located on natural (e.g. gorges) or artificial (e.g. ruins, old deserted buildings) structures by barn owls, avoiding the use of nest boxes. Thus, parental individuals could more naturally select the nesting sites in relation to the environmental features (e.g. habitat structure, prey availability), rather than because of the presence of a nest box.

To minimise the effect of manipulation on chicks, blood samples and morphometric data were taken once they had a body mass ≈300 g (20–25 days of age). This approximate body mass and age was selected because chicks are at their highest growth rate (Taylor 1994), and consequently body condition and nutritional status are better assessed. Thus, data collection was conducted on chicks from mid-May to mid-June 2019. Chicks were covered with a blanket to minimise stress. Blood samples (0.5–0.8 mL) were extracted using heparinised syringes from the brachial vein of each chick. Blood samples were transferred to heparinised Eppendorf vials and transported in a cooler to the laboratory within the day of collection. Blood was extracted at 12:00 solar time to ensure that a similar period had elapsed among all the assessed chicks since the last meal during the previous night. This helps to control such a source of variation in blood parameters. After blood extraction, the wing and the tail of every chick were measured with a ruler (±1 mm). The head (bill-nape distance), the depth of the bill (culmen) and the length of the tarsus (tarsometatarsus) were measured with a digital calliper (±0.01 mm). Finally, the chicks were weighed with a spring balance (±1 g). The same person (D. Almeida) took all the measurements to control the error in data collection. Field procedures and animal manipulation complied with all regulations of Europe and Spain (a specific License was granted for Scientific Field Research in Madrid County) and they were carried out by trained personnel (D. Almeida) to avoid any adverse effect on barn owl chicks. After returning individuals to their nests, the number of chicks was recorded. Pellets were collected from nests and below them. Only whole pellets were collected to ensure the least time since production and to avoid double counts of the same prey. Moreover, the size of the pellets was selected to ensure that they all came from the chicks, as they have a smaller diameter than those from adults. For this selection, pellet production was previously monitored at each nest to determine the size of the adult pellets (with a similar diameter over time, >23 mm). During this study, the smallest pellets (i.e. from the chicks) increased over time, but they were never as large as those from adults (<23 mm in diameter). At each nesting site and within 4 previous days to the chick manipulation, 10–15 pellets were collected. This is considered to be an adequate number to assess the main species present in the diet (Bond et al. 2004, ≥10 pellets after applying cumulative occurrence plots on prey species). Pellets from each nest were separately labelled and refrigerated to prevent decomposition whilst in transit to the laboratory, where they were frozen (−20ºC). Geographical coordinates were recorded with a GPS to subsequently characterize each nest according to a set of habitat variables obtained after data processing with GIS (see 'Data Analyses' below).

Laboratory procedures

On arrival at the laboratory, blood samples were centrifuged (7000 rpm, 6 min) to remove the plasma, then stored at −80ºC until the biochemistry analyses. Four blood parameters were assessed: glucose, total proteins, triglycerides and alkaline phosphatase. These four parameters were chosen as they are well known to determine nutritional status in birds (Dawson & Bortolotti 1997, Williams et al. 1999). In particular, glucose and protein concentrations in blood are often elevated in early stages of development, as they are related to anabolism (growth). Triglycerides are indicative of the energy reserve and they are not usually used unless the primary source of cellular energy (glucose) is low. Regarding alkaline phosphatase, abnormally low levels of this enzyme (hypophosphatemia) may be associated with malnutrition in birds, due to the decrease of substrates for dephosphorylation from the digestive process. The four blood levels were determined by means of enzymatic-colorimetric methods (commercial testing kits, Sigma Diagnostics). Specifically, for total plasma proteins, serum bovine albumin was used as a standard.

After defrosting, the pellets were disaggregated in soapy water with tweezers and prey remains were extracted. Diagnostic hard structures (bones, hair, feathers, exoskeleton of insects) were inspected under a dissecting microscope (40×) to identify the prey categories consumed by chicks and to determine the minimum number of individuals of each prey category per pellet (e.g. skulls, Torre et al. 2015). Prey species were identified to the lowest possible taxonomic level following Muñiz et al. (1995). Particular body masses were assigned to each prey category, according to the average mass of specimens from collections and other information available in the literature (e.g. Muñiz et al. 1995).

Data analyses

Data for habitat variables were generated by GIS (ArcView 3.3) using the geographical coordinates of the nests and layers of land use and vegetation (15 categories at 25 m resolution, CORINE Land Cover 2018). Specifically, separate layer categories were: mixed/deciduous woodland, coniferous woodland, arable cereal crops, arable horticulture, arable set-aside, improved grassland, set-aside grassland, neutral grassland, calcareous grassland, acid grassland, wetlands, inland water, bare ground, suburban and continuous urban. Additional data layers were also used to generate variables of relief and distance (see below). A circular area of 1 km radius was established around the nests (approx. 3 km^2), as it is considered to be the average size of foraging area used by barn owls during the breeding season (Taylor 1994). According to Martínez & Zuberogoitia (2004), nine habitat variables were selected in the surroundings of the nests for being potential determinants in the feeding of barn owl chicks: tree cover (height >200 cm, %), bush cover (100–200 cm, %), pasture cover (<100 cm, %), non-vegetated cover (urban area, %), relief (number of 1-m height contour cuts by four 100 m lines starting from the centre of the area in directions N, S, E and W), altitude (m a.s.l.), distance to aquatic environment (m), distance to urban area (m) and distance to road (m).

Three overall dietary indices were calculated and expressed as a percentage for each prey category: 'occurrence' (%Oc., frequency of pellets in which a particular prey category occurred relative to the total number of pellets), 'number' (%N, frequency of individuals of a particular prey category relative to the total number of individuals of all prey categories) and 'mass' (%Mass, frequency of the body mass of a particular prey category relative to the total body mass of all prey). Prey 'richness' (number of different prey categories, *S*) and prey 'diversity' (Shannon index, *H'*) were also calculated for each nest.

Canonical Correspondence Analysis and Redundancy Analysis were performed to describe the main sources of variation, although they did not provide an appropriate understanding of these particular data-sets on habitat and diet. Alternatively, habitat and dietary (i.e. %Mass) data were analysed using Principal Component Analysis (PCA). The two first components (PC1 and PC2) with eigenvalues >1 were extracted for habitat (H) and diet (D), respectively. Orthogonal varimax rotation was used to maximise the explained variance. For further analysis of feeding habits, Detrended Correspondence Analysis (DCA) was used on dietary data to describe the main sources of variation and the associations between nesting sites and prey categories. DCA avoids both the 'arch' and 'edge' effects on the gradient from a simple Correspondence Analysis.

Path and Multiple Regression Analyses were used to assess the relationships between the response variables (i.e. brood size, body condition and nutritional status) and the predictor variables (i.e. habitat structure and dietary traits). However, these statistical approaches did not provide clear patterns. Alternatively, the relationships between nesting habitat structure (i.e. PCs-H) and dietary traits (prey richness, prey diversity and PCs-D) were assessed by means of partial correlations (*pr*). Similarly, brood size and nutritional status (blood parameters) were also related to habitat (PCs-H) and dietary (PCs-D) gradients. These sets of correlations were performed after controlling for the effect of chick body mass (covariate). Particularly for chick body mass, this variable was also related to the PCs-H and PCs-D by *pr*, while controlling for size. To control for this 'size effect' and thus truly to assess body condition, a PCA was conducted with varimax rotation to objectively reduce the original variable set of size measures (i.e. the five types of morphometric data, see above) by extracting the first PCs with eigenvalues >1. These new factors typically explain most of the variance and can be used as multivariate indices of overall body size, namely 'structural size', which is preferable to univariate measures (Freeman & Jackson 1990). The PCs of 'structural size' were considered as covariates of the body mass of the chicks, as this is a better statistical approach to avoid bias while controlling for size effects (García-Berthou 2001).

Generalised Linear Mixed Models (GLMM) were previously used to assess the effect of 'rank in the brood hierarchy' as random factor (e.g. Blanco et al. 2004). The statistical models indicated no significant effect of this factor on the response variables. Furthermore, outliers were not detected, i.e. chicks in either extremely good or extremely poor body condition and nutritional status. Consequently, to avoid pseudo-replication, each nest was considered as one replicate ($n = 20$), with average data from the chicks and pellets being calculated. Data were transformed by using $\ln(x + 1)$. Particularly for percentage data, logit-transformation was used, since Warton & Hui (2011) recommend this method to analyse proportions in ecology research as an alternative to arcsine square root transformation. Assumptions of normality of distributions and homogeneity of variances were verified through Shapiro–Wilk and Levene's tests, respectively. All statistical analyses were performed with R ×64 v3.6.3 (R Development Core Team 2020). The significance level was set at $\alpha = 0.05$. Values reported in results are means ± SE.

Results

Habitat structure

The two first PCs of habitat variables (i.e. PC1-H and PC2-H) at the nesting sites explained a high percentage of variance (39% and 21% of the total variation, with eigenvalues = 3.47 and 1.93, respectively). Relief, distance to road, percentage of tree cover and percentage of bush cover were the variables that showed the strongest positive correlations with PC1-H (Factor Loadings: $r = 0.84$, 0.79, 0.76 and 0.72, respectively), while the percentage of pasture cover was negatively correlated with this axis ($r = -0.91$). Distances to urban area and aquatic environment were positively correlated with PC2-H ($r = 0.74$ and 0.58, respectively), whereas the percentage of non-vegetated cover was negatively associated to this second component ($r = -0.75$) (Fig. 1). The PC1-H axis represented a gradient of vegetation type, from pasture to forest environments in mountains, away from human influence (roads). The PC2-H represented a gradient of urban influence. Thus, in the two-dimensional graphic, the coordinates of the two first PCs-H allowed clustering of the nests according to the following types of structural habitats (Fig. 1): 1) 'mountainous forest' (rhombi, clustered in axis 1); 2) 'urban' (circles, clusterered in both axes); 3) 'crop under low urban influence' (triangles, clustered in both axes); and 4) 'crop under moderate urban influence' (squares, clustered in both axes).

Feeding habits

The most important prey for the three dietary indices was the house mouse *Mus musculus* (L., 1758), with an occurrence of more than 35% in the pellets and over a fifth and a quarter of the total prey as number and ingested mass, respectively (Table 1). Cabrera's vole *Microtus cabrerae* (Thomas, 1906) was the second type of prey with higher occurrence and ingested mass (about 20% in both cases), also being important in number (>10%). The greater white-toothed shrew *Crocidura russula* (Hermann, 1780), the Mediterranean pine vole *Microtus duodecimcostatus* (Sélys-Longchamps, 1839) and the

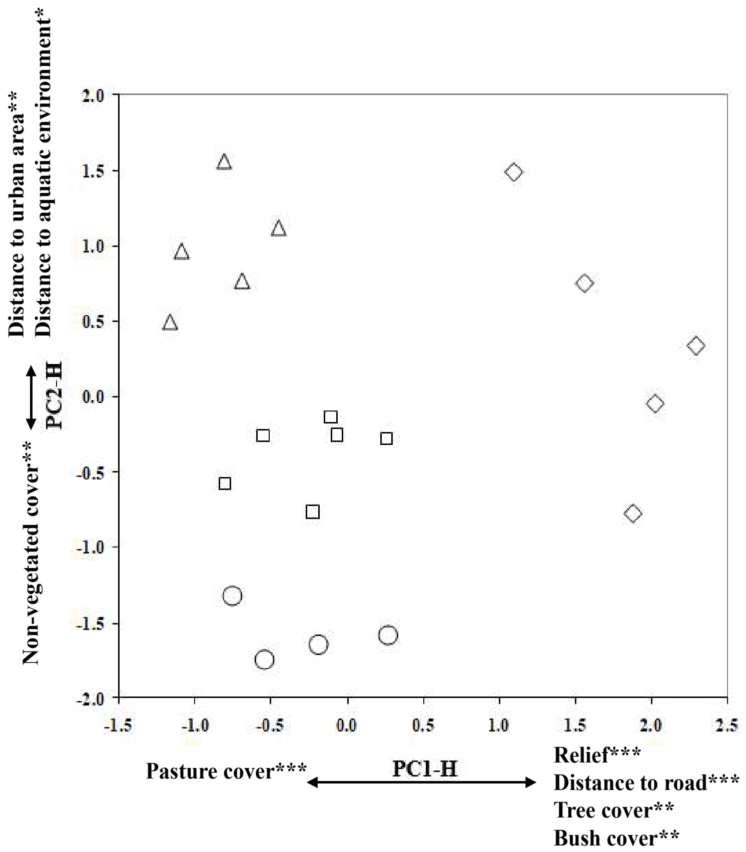

Fig. 1. PCA biplot for nesting sites ($n = 20$) of barn owl *Tyto alba* (i.e. PC1-H and PC2-H). Symbols refer to the four main types of nesting habitat (see details in Data analyses): rhombus – 'mountainous forest', circle – 'urban', triangle – 'crop under low urban influence' and square – 'crop under moderate urban influence'. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$.

Table 1. Diet of barn owl *Tyto alba* chicks ($n = 231$ pellets) from the study nests. Percentages of occurrence (%Oc., and absolute number of occurrences), number (%N, and absolute number of prey) and ingested mass (%Mass) are presented for every prey category.

Prey category	**%Oc.**	**%N**	**%Mass**
Orthoptera	4 (9)	3 (23)	1
Coleoptera	13 (30)	6 (44)	<1
Alaudidae	5 (12)	3 (22)	2
Common blackbird *Turdus merula*	1 (2)	1 (8)	<1
House sparrow *Passer domesticus*	12 (28)	8 (61)	4
European goldfinch *Carduelis carduelis*	4 (9)	2 (15)	1
Greater white-toothed shrew *Crocidura russula*	17 (39)	14 (105)	4
Etruscan shrew *Suncus etruscus*	9 (21)	6 (46)	1
Cabrera's vole *Microtus cabrerae*	19 (44)	11 (83)	21
Mediterranean pine vole *Microtus duodecimcostatus*	16 (37)	9 (69)	14
Brown rat *Rattus norvegicus*	6 (14)	3 (21)	9
House mouse *Mus musculus*	36 (83)	21 (158)	26
Algerian mouse *Mus spretus*	16 (37)	8 (60)	10
Wood mouse *Apodemus sylvaticus*	8 (18)	5 (38)	7

Algerian mouse *Mus spretus* (Lataste, 1883) were other important prey (Table 1). Among birds, the house sparrow *Passer domesticus* (L., 1758) was the most consumed species; whereas Alaudidae, common blackbird *Turdus merula* (L., 1758) and European goldfinch *Carduelis carduelis* (L., 1758) were of lesser importance in the diet of the barn owl chicks. Regarding invertebrates, Coleoptera (families Geotrupidae and Scarabaeidae) occurred in about 13% of the pellets, although they were not an important resource in terms of ingested mass (<1%).

The first two dimensions of DCA explained

34% and 28% of the total variation (eigenvalues = 0.52 and 0.44), respectively. The DCA biplot displayed clustering of several nests and food categories (Fig. 2): 1) nests in 'mountainous forest' habitats associated with Coleoptera, greater white-toothed shrew, Etruscan shrew *Suncus etruscus* (Savi, 1822), Cabrera's vole and wood mouse *Apodemus sylvaticus* (L., 1758); 2) nests in 'urban' habitats, where common blackbird and brown rat *Rattus norvegicus* (Berkenhout, 1769) dominated the diet; 3) nests in 'crops under low urban influence', where the presence in the diet of Algerian mouse and Mediterranean pine vole was frequent; and 4) nests in 'crops under moderate urban influence' associated with house sparrow and house mouse.

Prey richness was $S = 5.7 \pm 0.3$ (range: 4–8) and prey diversity was $H' = 2.25 \pm 0.06$ (range: 1.68–2.61). None of these indices were associated with the gradient of urban influence (PC2-H). However, prey diversity was significantly higher in the nests characterized by high values of PC1-H (well-developed vegetation in mountainous habitats) ($pr = 0.62$, $P = 0.010$). The prey richness also displayed the same trend of variation with PC1-H, although this relationship was marginally non-significant ($pr = 0.52$, $P = 0.057$).

The first two components of PCA on dietary data (i.e. PC1-D and PC2-D) explained a high proportion of variance (32% and 28% of the total variation, with eigenvalues = 3.24 and 2.83, respectively). The house mouse and the brown rat showed significant ($P < 0.05$) and positive factor loadings for PC1-D ($r = 0.79$ and 0.63, respectively); while the wood mouse, the Etruscan shrew, Coleoptera and the Cabrera's vole were negatively correlated with this first axis ($r = -0.86, -0.78, -0.58$ and -0.57, respectively). House sparrow was positively correlated to PC2-D ($r = 0.67$), while Algerian mouse and Mediterranean pine vole showed highly significant ($P < 0.001$) negative relationships ($r = -0.92$ and -0.89, respectively). PC1-D represented a gradient in the use of prey, from those inhabiting human-influenced environments (human-associated Muridae) to prey typical of wild environments, such as the wood mouse, the Etruscan shrew and the Cabrera's vole. PC2-D represented a gradient of anthropic influence on the consumption of prey associated with crop-land environments, from a high consumption of house sparrow (higher human association) to an increase in the sort of prey typical of croplands under low urban influence, such as the Algerian mouse and the Mediterranean pine vole.

Significant negative correlations were found between PC1-H and PC1-D ($pr = -0.71$, $P = 0.005$), and also between PC2-H and PC2-D ($pr = -0.74$, $P = 0.003$). Thus, the nests located in mountainous habitats with well-developed vegetation cover were associated with a diet based on wild prey, while chicks in nests located in areas with sparse vegetation were chiefly fed on house mouse and brown rats. According to the negative correlation between PC2-H and PC2-D, the barn owl chicks that had grown in urban-influenced environments had a diet with a high proportion of house sparrows and, as the nest location moved away from these habitats, they included in their diet rodents associated with crops under a low urban influence, mainly Algerian mice and Mediterranean pine voles.

Productivity and nestling quality

Data from 56 chicks were collected at approximately 20–25 days of age and this number was the same as per fledglings (i.e. no mortality amongst the offspring) at approximately 1.5 months after hatching. Brood size was 2.8 chicks ± 0.3 (range: 1–5). PC1-D was positively correlated with brood size ($pr = 0.55$, $P = 0.040$). Thus, the nests with more chicks consumed a higher proportion of human-associated Muridae. PC2-H was negatively correlated to brood size, although this relationship was marginally non-significant ($pr = -0.52$, $P = 0.054$). As low values of PC2-H were typical of high urban influence (Fig. 1), such a negative relationship implies that brood size was larger next to urban areas.

Body mass was 323.0 g ± 3.5 (range: 287–362). To control for the effect of size, the two first PCs with eigenvalues 2.56 and 2.07 (49% and 32% of explained variance, respectively) were used as indices of 'structural size'. The relationship between the corrected body mass and PC1-H was significantly positive ($pr = 0.68$, $P = 0.007$). This means that the chicks displayed better body condition in environments characterized by mountainous forests. In relation to diet, a negative correlation was found with PC1-D ($pr = -0.54$, $P = 0.046$). Thus, the nests exhibiting chicks with better body condition were related to a low proportional consumption of human-associated Muridae.

Glucose level was 0.81 mg $\mathrm{mL^{-1}}$ ± 0.13 (range: 0.31–1.77). Total protein level was 53.68 µg $\mathrm{mL^{-1}}$ ± 3.13 (range: 39.03–84.58). Triglycerides level was 0.73 mg $\mathrm{mL^{-1}}$ ± 0.04 (range: 0.41–0.95). Alkaline phosphatase level was 50.28 U $\mathrm{L^{-1}}$ ± 6.07 (range: 20.63–104.09). PC1-H was positively correlated with protein ($pr = 0.68$, $P = 0.008$) and alkaline phosphatase ($pr = 0.66$, $P = 0.011$) levels. This positive correlation was similar for glucose level, although marginally non-significant ($pr = 0.52$, P

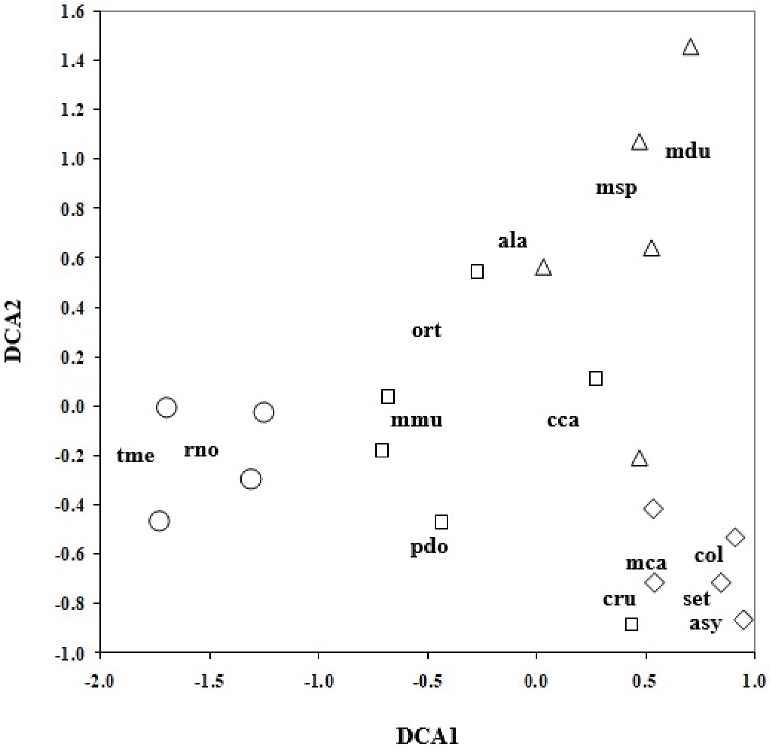

Fig. 2. DCA biplot for nesting sites ($n = 20$) and prey categories of barn owl *Tyto alba* chicks. Symbols refer to the four main types of nesting habitat (see PCA results on structural habitat): rhombus – 'mountainous forest', circle – 'urban', triangle – 'crop under low urban influence' and square – 'crop under moderate urban influence'. Prey categories are: ala – Alaudidae, asy – *A. sylvaticus*, cca – *C. carduelis*, col – Coleoptera, cru – *C. russula*, mca – *M. cabrerae*, mdu – *M. duodecimcostatus*, mmu – *M. musculus*, msp – *M. spretus*, ort – Orthoptera, pdo – *P. domesticus*, rno – *R. norvegicus*, set – *S. etruscus*, tme – *T. merula*.

= 0.059). Thus, the nests proximal to mountainous habitats with well-developed vegetation and away from roads had chicks with higher levels of these three blood parameters (i.e. protein, phosphatase and glucose). Regarding diet, the relationships were negative between PC1-D with protein ($pr = -0.62$, $P = 0.020$) and glucose ($pr = -0.60$, $P = 0.021$) levels. Therefore, the values of these two parameters were lower in chicks fed with a high proportion of human-associated Muridae. The triglycerides level was the only blood parameter that was not clearly correlated with any PC (overall $P > 0.10$).

Discussion

In this study, the diet of barn owl chicks was observed to vary greatly in terms of taxonomy (i.e. including insects, birds and mammals), although other prey types also occur in Europe, such as amphibians, reptiles and bats (e.g. SZÉP et al. 2021). This plasticity in the foraging behaviour is due to the capacity of barn owls to widen their trophic niche to secondary groups (e.g. amphibians, reptiles or birds) where and when availability of their optimal prey (micromammals) is low (GUERRA et al. 2014). TAYLOR (1994) observed that, in Mediterranean ecosystems, rodents of the family Muridae are the main input of mass to barn owl diet, whereas Cricetidae are the main input in the rest of Europe. These results are similar to those from the present study, where four species of Muridae were found in the diet and only two species of Cricetidae.

TETA et al. (2012) showed a clear association between habitat features and diet composition of barn owls, with a higher consumption of human-associated Muridae in urban areas and a higher consumption of native rodents (wild prey) away from these habitats. A similar gradient in prey consumption was found in this study. Furthermore, support was found for the first hypothesis concerning the relationship between habitat heterogeneity and dietary diversity. Structurally more heterogeneous environments ('mountainous forest' nesting sites) promoted a higher prey richness and diversity in the diet of chicks. This result is most likely related to the increase of different prey groups in the margins of habitat mosaics, whereas the decrease in dietary diversity observed in pasture environments –mainly monoculture crops–, is due to the fact such habitats are usually exploited by a lower number of prey species (McCollin 1998, Saufi et al. 2020). Stability in refuge conditions and food supply are two main aspects of assessing the habitat quality and consequently, of estimating its potential as a breeding area for barn owls (Salvati et al. 2002, Bond et al. 2004, Hindmarch et al. 2012). The present results show that larger brood sizes were more frequent in areas subject to urban influence, since barn owls display a high human tolerance and opportunistic behaviour (Salvati et al. 2002). Additionally, competitors for food or nesting habitat (e.g. tawny owl *Strix aluco* (L., 1758)) and potential predators (e.g. common genet *Genetta genetta* (L., 1758)) are less tolerant and thus, scarcer in anthropic environments (Bunn et al. 2010). Moreover, and according to the second hypothesis, larger brood sizes were linked to increased consumption of human-associated prey (house mouse and brown rat). Detection of these Muridae species by barn owls may be easier in urban environments (less refuge due to lack of vegetation) and also, their anti-predatory strategies may be of low efficiency (noisier prey) (Saufi et al. 2020). Human-associated Muridae can take advantage of the more stable source of refuge and food in urban habitats to achieve high reproductive rates, which can be maintained over time (several large litters per year). Consequently, Martínez & López (1999) suggested that these rodent species in urban and sub-urban environments represent a trophic resource constant enough to contribute to large brood sizes of barn owls in Mediterranean ecosystems. Furthermore, Muridae populations do not show such large inter-annual fluctuations compared to other rodent groups (e.g. Cricetidae, see Klemola et al. 2002).

Barn owl chicks raised in habitats with well-developed vegetation and away from roads exhibited better body condition and nutritional status (i.e. overall good nestling quality). Roads have been highlighted as a disruptive factor of particular importance for Iberian barn owl populations because of two main mechanisms: 1) habitat fragmentation; and 2) juvenile mortality (Borda-de-Água et al. 2014). Therefore, roads should be located far from the most sensitive areas for barn owl breeding and designed to minimise traffic-related mortalities (e.g. the addition of high vegetation at both road-sides to force birds to fly up and over them). Moreover, diet was more diverse and based on wild micromammals in mountainous habitats. These prey species are probably more nutritious and efficiently digested, enabling a higher rate of weight gain, as the barn owl may be adapted to their consumption (e.g. co-evolutionary history with wild prey). Conversely, human-associated Muridae are not food items of good quality, since they are related to low levels of nutrients, as previously observed by Clum et al. (1997) in captive raptors. Thus, the results of the present study highlight two well-defined breeding strategies dependent on habitat structure, specifically a high number of 'low-quality' nestlings *vs.* a low number of 'high-quality' nestlings. This is a typical trade-off in the reproductive effort under natural conditions (e.g. Smith et al. 1989). In particular, Tinbergen & Boerlijst (1990) and also Magrath (1991), assessing passerine species as biological models, demonstrated that the body mass of chicks is positively related to juvenile survival and this mass is a better correlate than brood size to estimate the probability of future breeding and effective contribution to population recruitment, i.e. biological fitness (Clutton-Brock 1988). In the barn owl, a better body condition of chicks provides a higher capacity to overcome the first winter, the season of highest juvenile mortality in this species (Massemin & Handrich 1997). Moreover, Roulin (2002) observed that juvenile survival in the barn owl decreased in the largest brood sizes with the increase of social stress by rivalry among siblings, probably due to the strong size hierarchy as a result of asynchronous hatching (Mock & Parker 1997). However in the present study, this effect (i.e. brood hierarchy) was not observed in the response variables representing nestling quality, i.e. body condition and nutritional status (see 'Data Analyses' above).

An interesting finding of this study was the lack of association between the triglycerides level and both habitat features and diet composition. This could be related to the early developmental stage of individuals. As chicks must grow fast, glucose is the main source of energy for anabolism, since it is a molecule with high energy content and is easy to transport and metabolise (highly water-soluble). This nutrient, along with structural components (proteins), are regularly provided by parents, with chicks investing the majority of nutritional input in rapid growth and only a small amount as fat reserves (Starck & Ricklefs 1998). As an example, Durant et al. (2008) found an excess of body mass in barn owl chicks before fledging because of water reserves rather than energy (fat) reserves to overcome the scarcity of water resources.

The present paper highlights the usefulness of assessing different indicators (ecological, morphological and physiological traits) to provide more accurate insights into the productivity and nestling quality in the barn owl, as the effect of environmental factors (habitat structure, prey availability) on this species can be highly variable (e.g. Milchev 2015). This information may be applied by policy-makers and environmental managers for the conservation of Iberian barn owl populations.

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