

Dietary selectivity mediates niche overlap and interspecific competition in a reedbed warbler community in southern Europe

S. R. DAVIES,^{*,†,1}  I. P. VAUGHAN,¹  R. J. THOMAS,¹ P. JOSA,² S. BATEMAN POSSE³ & W. O. C. SYMONDSON^{1,‡}

¹*School of Biosciences, Cardiff University, Cardiff, CF10 3AX, UK*

²*Institut Català d'Ornitologia, Museu de Ciències Naturals de Barcelona, Barcelona, 08019, Spain*

³*Grup de Natura Freixe, Reserva Natural de Sebes, Flix, 43750, Tarragona, Spain*

Sympatric species are expected to use resources differently along one or more ecological dimensions. This can be complex when there is partial separation along multiple dimensions, such as where bird species exhibit similarities in habitat use and diet. We used DNA metabarcoding to examine the diet of three sympatric warblers: the migratory Eurasian Reed Warbler *Acrocephalus scirpaceus* and Great Reed Warbler *Acrocephalus arundinaceus*, and the resident Cetti's Warbler *Cettia cetti*, breeding in reedbeds in Catalonia, Spain. Although the diets of the three species partially overlapped, each consumed some distinct prey taxa, and/or key species at differing frequencies. Compared to the Eurasian Reed Warbler, the Great Reed Warbler consumed a greater proportion of larger prey, while Cetti's Warbler consumed a greater proportion of terrestrial species. Together, these results indicate a degree of dietary niche separation between warbler species, via species-specific prey selectivity, which may explain their coexistence in Catalanian reedbeds. Our findings have implications for understanding future competitive pressure and coexistence of reedbed warblers under the dual, connected threats of climate change and global invertebrate decline.

Keywords: Cetti's Warbler, dietary segregation, Eurasian Reed Warbler, Great Reed Warbler, niche overlap.

Resource partitioning theory predicts that sympatric species should exhibit differences in resource use along one or more ecological gradients (MacArthur 1958, MacArthur & Levins 1967, Schoener 1974b). This has been demonstrated in a wide range of organisms including birds (Lack 1976, Hiron *et al.* 2006), bats (Salsamendi *et al.* 2012), lizards (Pianka 1974) and arthropods (Albrecht & Gotelli 2001), providing insights into competition,

coexistence and food web structure. Interspecific competition between sympatric species may differ across the focal species' range, either intensifying or relaxing in response to environmental conditions (Schoener 1974a, Wiens 1977), or in the presence or absence of additional competitors (Diamond 1973, Freeman *et al.* 2016). For sympatric bird species, dietary niche differentiation can reduce competition for food resources and facilitate the coexistence of otherwise ecologically similar species, which in turn supports biodiversity (MacArthur & Levins 1967, Schoener 1974a, Kent & Sherry 2020).

Understanding how sympatric species partition prey resources requires detailed knowledge of diets. One of the greatest barriers to dietary studies is the lack of taxonomic resolution from traditional 'hard-part' analysis of prey remains

[†]Present address: Wetland Evidence Department, WWT Slimbridge, Gloucestershire, GL2 7BT, UK

*Corresponding author.
Email: sarah.r.davies90@gmail.com

[‡]This author passed away during the preparation of the manuscript.

(Pompanon *et al.* 2012). For many vertebrates, DNA metabarcoding using faecal material or gut contents allows greater taxonomic resolution and detectability of dietary items (Valentini *et al.* 2009, Shokralla *et al.* 2012, Bohmann *et al.* 2014), resolving the subtle but important differences in prey utilization between potential competitors (Krüger *et al.* 2014, Crisol-Martínez *et al.* 2016, Zurdo *et al.* 2023a, 2023b). This approach is often applied to bird studies (Hoenig *et al.* 2022), to investigate dietary partitioning in avian communities (e.g. Trevelline *et al.* 2018b, Spence *et al.* 2021, Zurdo *et al.* 2023a, 2023b) and intraspecific variation among age or sex classes (e.g. Davies *et al.* 2022, Stenhouse *et al.* 2023).

Warbler species with similar breeding habitat requirements frequently co-occur in the same wetlands (Martínez-Vilalta *et al.* 2002). This suggests ecological segregation, either directly through differences in foraging behaviour or microhabitat use (Lovette & Hochachka 2006), or indirectly from morphological differences in body size, bill size or bill shape (MacArthur 1958, Pigot *et al.* 2018). The reedbed ecotones of southern Europe commonly support three co-occurring warbler species: the Eurasian Reed Warbler *Acrocephalus scirpaceus*, the Great Reed Warbler *A. arundinaceus* and Cetti's Warbler *Cettia cetti*. While the Eurasian Reed and Great Reed Warbler are congeneric Afro-Palearctic migrants, the latter being approximately 2.7 times larger in body mass than the former, Cetti's Warblers are non-migratory residents of a separate family: the Cettiidae (Cramp & Brooks 1992).

Dietary studies based on 'hard part' analysis demonstrate that these three species consume a wide array of aquatic, semi-aquatic and terrestrial prey (Cardenas *et al.* 1983, Molina *et al.* 1998, Ferreira-Carballal *et al.* 2017, Ientile *et al.* 2022). These analyses typically identify arthropods (particularly morphologically cryptic groups) to the level of order or above, and potentially underestimate dietary segregation and dietary niche breadth at the level of prey species (Symondson 2002, Pompanon *et al.* 2012). They are also biased towards larger, harder-bodied prey (identified by wing fragments and mandibles) that survive digestion (Sherry *et al.* 2016) and may fail to detect or identify easily digestible or softer-bodied prey such as caterpillars and flies, leading to underrepresentation of these groups (Pompanon *et al.* 2012, Nielsen *et al.* 2018, Ientile *et al.* 2022).

Several factors may facilitate ecological segregation between the three species. First, reedbeds comprise several microhabitats, often arranged along a successional gradient from open water to the surrounding terrestrial habitats. Differences in microhabitat use by reedbed warblers are widely recognized (Bibby & Green 1983, van der Hut 1985, Leisler & Schulze-Hagen 2011, Koma *et al.* 2021), with Great Reed Warblers preferring more open areas of reedbed with thicker reed stems, near open water zones and Eurasian Reed Warblers using denser stands of thinner-stemmed reeds, often in less productive areas (Dyrce 1981, Leisler 1981, Poulin *et al.* 2002, Mérő *et al.* 2015, Koma *et al.* 2021). Cetti's Warblers primarily inhabit areas of scrub that are often adjacent to, and admixed with, areas of reedbed (Bibby & Green 1983, Balança & Schaub 2005).

Secondly, Eurasian Reed and Great Reed warblers are a widely cited example of asymmetric competition between closely related species of different body sizes, with exploitation competition (an indirect, negative interaction that occurs through use of a shared, limited resource (MacArthur & Levins 1967)) and predator-mediated apparent competition (an indirect, negative interaction mediated by a shared predator (Holt 1977)) thought to be among the primary drivers of reproductive success in both species (Hoi & Winkler 1994, Schaefer *et al.* 2006).

Finally, in general, resident species may monopolize the most profitable trophic resources year-round, thus obliging the arriving migrants to widen their dietary breadth and consume less optimal or smaller prey (Poulin & Lefebvre 1996). In reedbed systems, it follows that resident Cetti's Warblers may be more selective than migrant warblers. However, this mechanism may be outweighed by the differences in microhabitat use by Eurasian and Great Reed Warblers. For example, the more terrestrial habitat choice of the Cetti's Warbler may allow for a broader diet than expected if their foraging habitat overlapped entirely with that of Eurasian and Great Reed Warblers.

Conversely, other mechanisms may reduce ecological segregation. Many arthropod prey are highly mobile between wetland microhabitats and available to consumers in each of those different habitats (Wilson 2010). Different foraging methods exhibited by sympatric species may still result in each species capturing the same prey species in the same or different microhabitats, leading

to greater overlap than might otherwise be expected (Poulin & Lefebvre 1996, Gordon *et al.* 2019, Navalón *et al.* 2019). Moreover, the general habitat associations of reedbed warbler species mask a degree of flexibility in nesting territories and foraging locations, challenging the idea that reedbed warblers are highly habitat-specific (Thomas 1984, Ceresa *et al.* 2016, Fontanilles *et al.* 2024). A degree of habitat overlap between conspecific species could be commonplace when foraging, driving higher dietary overlap when and where similar, abundant prey items are encountered and consumed.

In recent decades, Afro-Palaearctic migrants have experienced greater declines than resident or short-distance migrants (Vickery *et al.* 2014). Causes are multifaceted and exacerbated by climate change, for example, the degradation of habitat quality in breeding and non-breeding grounds, extreme weather (including drought in the Sahel) and changes to prey phenology driving trophic mismatches during chick-rearing, with specialists therefore more at risk than generalists (Di Cecco & Hurlbert 2022). Despite conservation efforts in Europe, evidence from a 30-year study suggests that networks of small, protected areas favour generalist wetland species with less specific ecological requirements over habitat specialists (Brambilla *et al.* 2020). Understanding the diets and feeding strategies of wetland insectivores, such as reedbed warblers (both resident and migratory), will help us to assess their vulnerability to the interacting threats of climate change and documented worldwide declines in arthropod abundance (Halsch *et al.* 2021, Wagner *et al.* 2021). We used DNA metabarcoding to characterize the diets of the three focal warbler species, across four reedbed study sites in Catalonia, Spain, over the 2018 breeding season. Few metabarcoding studies have so far assessed dietary composition, breadth and overlap in multi-species avian insectivore assemblages across multiple wetland sites and very few consider differential consumption of prey by size, or by functional group, for example, aquatic versus terrestrial prey (Trevelline *et al.* 2018a, 2018b, Hoenig *et al.* 2022). Addressing this gap will advance our ecological understanding of how passerines with similar life histories and habitat requirements select prey and avoid competitive exclusion in human-altered environments experiencing insect decline and climate change impacts.

We hypothesized that the three species partition prey resources, reducing dietary overlap. This will be reflected by low values of Pianka's measure of dietary overlap (O_{jk}), and interspecific differences in (i) overall diet composition (frequencies of individual prey items in the diet), (ii) the proportion of aquatic prey species in the diet (i.e. different prey functional traits) and (iii) the body size classes of consumed prey (i.e. different prey characteristics). As generalist insectivores, we expected that all three warbler species would show a broad dietary niche to reflect the diversity of arthropods, but we predicted significant differences in niche breadth among the three warbler species, driven by interspecific differences in foraging strategies and prey selection.

METHODS

Study locations

Five research sites were included in this study (Fig. 1), comprising four overall sites for analysis:

- i Mas del Matà, a wetland site forming part of the nature reserve Aiguamolls de l'Empordà (42°12'47.55"N, 3° 5'57.05"E) in northern Catalonia.
- ii The Llobregat Delta (comprising two connected reserves; Cal Tet and Remolar Filipines): (together; 41°17'44"N, 2°6'43"E): a series of coastal wetlands south of Barcelona. In this study, we often refer to Cal Tet as simply Llobregat Delta, as this was the principal site studied.
- iii Reserva Natural de Sebes (41°14'15.57"N, 0°30'39.49"E): a wetland nature reserve along the River Ebro in western Catalonia.
- iv Canal Vell Biological Station (40°44'34.61"N, 0°47'10.25"E): in the Ebro Delta Natural Park at the northern shore of the Canal Vell coastal lagoon, situated on the eastern coast of Catalonia.

Sites differed in topography, proximity to the coast, altitude and sub-climate, but comprised similar warbler assemblages.

Common Reed *Phragmites australis* reedbeds dominated large areas of all the sites. Reedbed zones were fringed by areas of herbaceous vegetation and scrub, largely composed of *Tamarix* and *Salix* species. In Sebes, an olive *Olea* plantation was at the edge of the scrub. In Canal Vell, lines

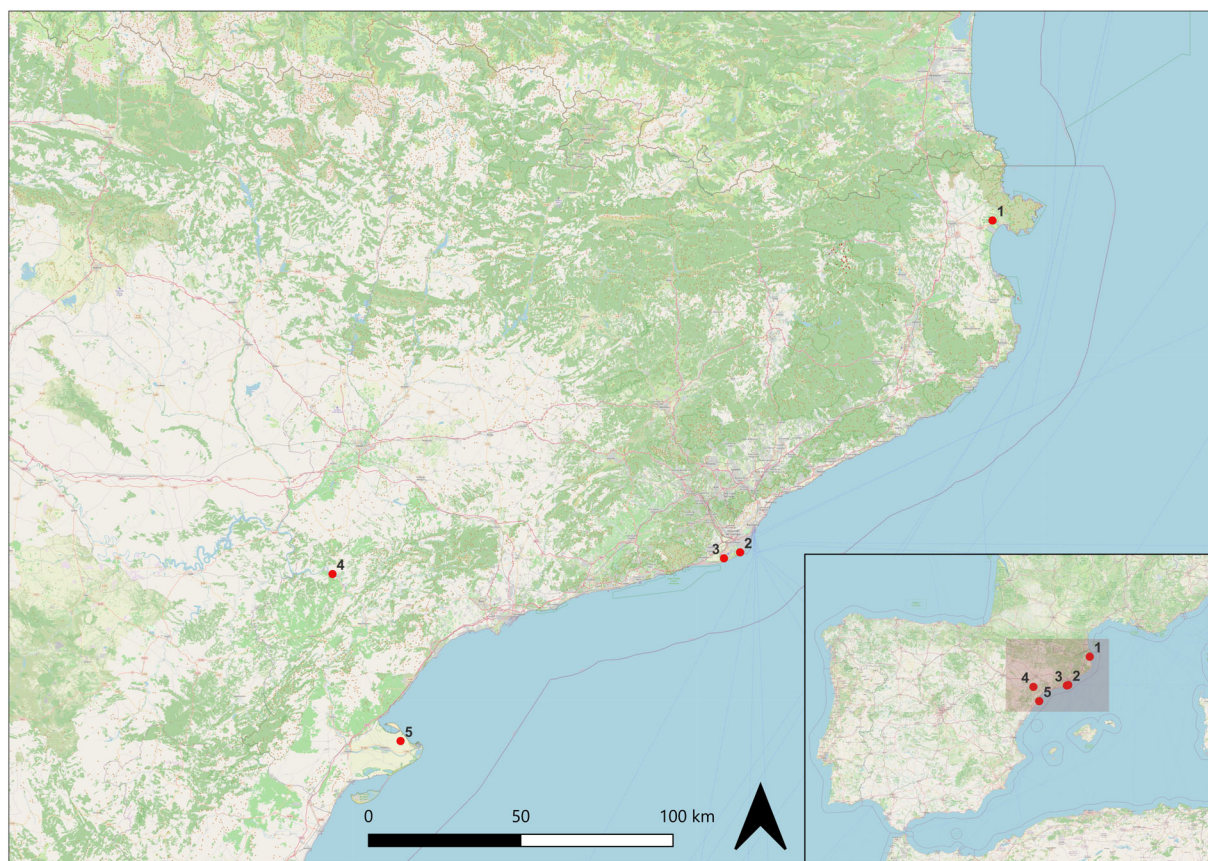


Figure 1. Map of Spain (insert) and Catalonia (main map), with the study area indicated in the shaded box of the insert. Numbered points indicate the location of the five study sites: 1. Mas del Matà (Aiguamolls de l'Empordà), 2. Cal Tet (Llobregat Delta), 3. Remolar Filipines (Llobregat Delta), 4. Reserva Natural de Sebes and 5. Canal Vell (Ebro Delta).

of the non-native figwort species *Myoporum tenuifolium*, planted in the early 1990s, were present throughout the site. The surrounding areas of the Canal Vell lagoon were composed mainly of rice *Oryza* fields.

Daily maximum temperatures in summer 2018 ranged from ~20°C to 35°C. Precipitation was low, with rainfall events becoming less frequent by mid-summer. The sites became increasingly hot and dry over the study period, with many aquatic habitats becoming desiccated by July.

Field methods

Faecal samples were collected from mist-netted birds that were ringed as part of SYLVIA, a monitoring programme led by the Catalan Institute of Ornithology (Institut Català d'Ornitologia, ICO).

Ringed sessions took place every 10–14 days, with an average of four visits to each site (range = 3–6 visits, total visits = 15) and four visits per month (range = 1–5 visits). At Canal Vell, Sebes and the Llobregat Delta, all faecal samples were collected between 11 May and 3 August 2018. At Aiguamolls de l'Empordà, sample collection took place over two consecutive days of bird ringing activity during the end of the spring migration in early May.

Mist-nets were erected through reedbed rides and along lines of adjacent scrub. While it was not possible to standardize mist-net captures completely, up to 21 nets of 6–18 m in length were set at each site (range = 8–21 nets). Mist-nets were opened at dawn (05:00–06:00 AM) and checked every 20 min. Birds were placed into clean, cotton holding bags while they awaited ringing and processing, so that faeces could be

collected. Birds were processed and released after 15 min in the bag regardless of whether defecation occurred. To prevent unnecessary stress to the birds, nets were taken down at 12:00–13:00 PM, or earlier if temperatures rose above 35°C.

The collection of faecal samples followed Davies *et al.* (2022). Faecal material was directly transferred from the clean bags into 2 mL tubes containing 100% ethanol in a ratio of 3:1 ethanol to faecal material. Samples were stored in a domestic freezer at –4°C until return to the U, where they were stored at –80°C. An average of 17.5 samples was collected per visit (Canal Vell = 18.3 (range 7–26), Llobregat Delta = 15.3 (range = 3–28), Aiguamolls de l'Empordà = 18.5 (range = 9–28), Sebes = 17.5 (range = 12–24)), giving an overall total of 265 samples available for analysis. Of these, 243 samples were taken forward for molecular analysis, the remaining 22 either containing insufficient material or representing a same-day, duplicate sample from the same bird recaptured on the same morning.

Molecular analysis and bioinformatics

DNA extraction, PCR, pooling, library prep and high-throughput sequencing (HTS) protocols were carried out as described in Davies *et al.* (2022) (see Data S1–S6). Arthropod DNA was successfully amplified in 194 samples. These were included in the final pool for HTS, alongside appropriate positive and negative controls, replicate samples and mock community mixes. Mock communities are composite samples containing DNA from multiple known taxa (in this case invertebrate species) in predefined ratios or compositions used to assess the reliability of metabarcoding-derived results (Iwazkiewicz-Eggebrecht *et al.* 2023).

The bioinformatics pipeline, including taxonomic assignment with BLAST+ (Camacho *et al.* 2009) and subsequent data clean-up steps, followed Drake *et al.* (2021) and Davies *et al.* (2022) (S4). We applied sequence thresholds to minimize the effects of 'tag-jumping'; that is, sequencing errors resulting from erroneous combinations of sample identity tags in HTS outputs (Schnell *et al.* 2015). We also removed sequences that were assigned to non-target taxa, as well as known contamination and artefacts (listed in S5).

Sequencing reads

After bioinformatic processing, 1 429 992 sequencing reads were recovered from the faecal samples. Of the total, 398 molecular operational taxonomic units (MOTUs) retrieved from the taxonomic aggregation step, 131 were considered potential prey items, after sequence cleaning. We retained a total of 1 226 502 diet reads, an average of 5988 ± 4589 sd diet reads per Eurasian Reed Warbler sample ($n = 137$), 8262 ± 4069 sd per Cetti's Warbler sample ($n = 26$ samples) and 7529 ± 4618 sd per Great Reed Warbler sample ($n = 26$ samples).

Overall, 189 warbler diet samples contributed to the final dataset (S7). The final number of reads for each unique prey taxon was converted to presence–absence data for each diet sample, in a diet matrix. We calculated frequency of occurrence (FOO), the proportion of samples in which each unique dietary taxon was detected, and expressed this as a percentage (% FOO) for each warbler species. All samples (including those from a small number of recaptured individuals) were included in overall % FOO calculations and comparisons.

Statistical analysis

For all dietary analyses, we used the presence/absence diet matrix of prey taxa $\times$ faecal sample ID. All statistical tests and models were run in R 4.0.4 (R Core Team 2021). To avoid pseudoreplication from inclusion of diet data from individual birds sampled more than once on different dates ($n = 8$), we randomly selected one sample from each recaptured bird (identified by their ring numbers) prior to carrying out statistical analyses. Sample sizes were reduced to 132 Eurasian Reed Warblers (five duplicate individuals removed), 26 Great Reed Warblers (no duplicates) and 23 Cetti's Warblers (three duplicates removed), giving a total of 181 samples (a breakdown per species by site and month is provided in S8).

Dietary overlap between pairs of warbler species was quantified with Pianka's overlap index (O_{jk}) (Pianka 1973); this is a measure of the degree to which two species use the same resources, scaling from 0 (no overlap) to 1 (complete overlap). Prey FOO matrices were generated for each warbler species pair, comprising two rows, one for each warbler consumer, where

columns represent prey dietary taxa. Each cell is a proportion, reflecting the number of times a given prey taxon was consumed by a warbler species, divided by the total number of diet samples for that warbler species.

A null modelling approach was used to test whether dietary overlap was significantly smaller than expected, consistent with our hypothesis, using the R package 'EcoSimR' (Gotelli & Ellison 2013). Randomization algorithm 3 (RA3) was run for 10 000 iterations on the frequency of occurrence matrix, randomizing prey species within each warbler (row), so that the same values of FOO were used but they were randomly reassigned to new prey identities. Niche breadth, here defined as the range and evenness of prey species consumed by each warbler, was kept constant; in other words, the distribution of FOO values in each row (warbler species) remained the same. RA3 thus simulated the range of interspecific overlap expected by chance, in the absence of dietary partitioning. Pianka's index was generated for each pair of warbler species, including observed values and simulated (expected) values, alongside a measure of variance.

To assess the sensitivity of each pairwise comparison among warbler consumers to unequal sample sizes, we used a parametric bootstrap of the input FOO matrices, with binomial resampling generating 500 replicate matrices for each warbler pair. To simulate a realistic matrix of prey occurrence, resampling was parameterized by observed prey frequency of occurrence p (the original FOO value in the observed diet matrix) and the sample size for each focal warbler species, n (Cetti's Warbler = 23, Great Reed Warbler = 26, Eurasian Reed Warbler = 132), reflecting sampling uncertainty. In each of the replicate matrices, every cell was given a new prey FOO value, the result of n Bernoulli trials (where n = sample size of the focal warbler species) each representing presence/absence of prey with probability of prey presence (p) determined by the observed FOO value for the equivalent cell in the original matrix. Each replicate bootstrap matrix was run in EcoSimR under RA3 for 999 simulations. We then checked if the distribution of Pianka's index overlap values and standardized effect sizes (SES) were centred on the observed overlap estimates across bootstrap replicates and whether the direction of null-model significance was consistent.

To investigate the degree of individual diet specialization among warbler species, we used the R package 'iNEXT' (Hsieh *et al.* 2016) to measure dietary niche breadth. We plotted coverage-based sampling curves, varying the diversity order parameter q to capture three measures of Hill numbers: $q = 0$ represents total prey species counts in diet samples (richness based), ignoring relative abundance or evenness; $q = 1$ represents the exponential of Shannon entropy (Shannon diversity), weighting prey species by their relative abundance (frequency) in the diet; and $q = 2$ represents a down-weighting of rare prey species (Simpson's diversity) and focusing on the dominant or core dietary niche. To account for the uneven sample sizes between warbler species, $q = 0$, $q = 1$ and $q = 2$ plots were examined at the same target sample coverage (SC), a measure of sample completeness (a proportion from 0 to 1 calculated by 'iNEXT'). This ensured a direct comparison of the dietary niche breadth of the three warbler species at equivalent levels of dietary sampling completeness.

Because warblers are insectivorous and expected to consume a large and diverse pool of available prey, SC was relatively low for each warbler species, particularly for Cetti's Warbler, the species with the fewest samples (SC = 0.55). SC curves showed that richness-based niche breadth ($q = 0$) and Shannon-based Hill numbers ($q = 1$) continued to increase steeply for Cetti's Warbler at low coverage. To minimize bias, core dietary niche breadth was estimated using the most conservative order: Simpson-based Hill numbers ($q = 2$) (see Data S11 for comparison of plots). Simpson-based core dietary breadth was expressed as the effective number of dominant prey taxa in the diet, standardized at 50% sample coverage (SC = 0.5), with uncertainty quantified via bootstrap confidence intervals. We confirmed the presence of significant differences in core dietary niche breadth between warbler pairs if the 95% confidence intervals (CIs) around the estimates did not overlap.

Differences in prey composition between the three species were tested at the species level using generalized linear models for multivariate abundance data, implemented using the *manyglm* function in the R package 'mvabund' (Wang *et al.* 2012). The response variable was the presence-absence diet matrix (warbler faecal sample $\times$ prey species) fitted using a binomial GLM with a 'cloglog' link function. We included 'Site'

as a fixed covariate in the model, as the number of study sites was small ($n = 5$ sites), but we treated and interpreted it as if it was a 'random' term (Gelman & Hill 2006). We used the *anova.manyglm* function to test for significant differences among warbler species using Monte Carlo resampling (resamp = 'montecarlo') and performed pairwise comparisons ('pairwise.comp' argument). Univariate testing identified which prey taxa had the strongest influence on the differences in diet composition between the bird species and sites (argument p.uni = 'adjusted'). To determine if dietary differences were consistent across taxonomic ranks, analyses were repeated with prey matrices collapsed to prey family and order levels (see Data S12). Broad differences in diet at the prey species level were visualized in two dimensions, by plotting a Non-metric Multi-Dimensional Scaling (NMDS) ordination with Jaccard dissimilarities, using the R package 'vegan' (Oksanen *et al.* 2018).

Information from the literature, specialist websites and invertebrate keys was collated to estimate the body length in millimetres of each prey species detected in the bird diets (S6). For consistency across taxa, we used reference imago body lengths to calculate the mean adult body length of all invertebrate prey species detected in each individual bird diet. Invertebrates were grouped according to whether they were terrestrial or had an aquatic life stage (classed as aquatic or semi-aquatic), and the proportion of prey species present from aquatic/semi-aquatic and terrestrial habitats was calculated for each individual sample.

GLMs were fitted to examine the difference between warbler species in (i) the average body length of prey species in the diet samples and (ii) the proportion of aquatic/semi-aquatic prey in diet samples weighted by species richness. As with our *manyglm* we included 'Site' as a fixed effect to improve model fit but interpret the results as if it were a random term. For the prey size model, a Gamma family and identity link function gave the best model fit. For the aquatic prey model, a binomial family and logit link function was first used, but overdispersion checks revealed a dispersion parameter greater than 1.5 ($\theta = 1.57$). Therefore, we used a quasibinomial model in which the variance was modelled with the parameter θ (rather than being set to 0). In these binomial/quasibinomial models, we used the argument 'weights' to weight the model by the richness of prey species in each faecal sample.

Pairwise comparisons of estimated marginal means (EMMs) between warbler species were carried out using the least-squares means function in the 'emmeans' package (Lenth & Piaskowski 2025), using the Tukey adjustment argument to control for multiple comparisons.

RESULTS

Warbler diet

In total, 30035 unique taxa were detected in the faeces of the three study species (Cetti's Warbler = 153 taxa, Great Reed Warbler = 78, Eurasian Reed Warbler = 221). Most taxa were identified to species level (85.1%) or genus level (12.5%) with a minority (2.4%) identified to family level or lower. The most frequently detected prey family was the Chironomidae, with a frequency of occurrence of 49.7% across the three warbler species (S7), followed by noctuid moths (24.9%), braconid wasps (20.6%), mosquitoes (19.6%) and clubionid spiders (19.6%). The top ranked prey consumed differed between the three warbler species, at both the prey species level (Table 1) and order level (Table 2; Fig. 2). Chironomids were consistently highly ranked in Eurasian Reed Warbler diet, whereas the top ranked prey in Great Reed Warbler diets and Cetti's Warbler diets were more diverse, with Great Reed Warblers often showing high frequency of large-bodied prey such as bush crickets (e.g. *Ruspolia nitidula*). In Cetti's Warblers, a wide range of species, from disparate arthropod orders (e.g. Psocoptera, Hemiptera, Araneae and Hymenoptera) ranked highly.

Dietary overlap

Pianka's measure of dietary overlap varied among species pairs (Table 3), but overlap was greatest between Eurasian Reed Warblers and Great Reed Warblers ($O_{jk} = 0.45$, $P = 0.002$), indicating a significantly greater niche overlap than expected by chance. Bootstrapped FOO matrices run under the simulation resulted in a median Pianka overlap value slightly lower than the observed overlap value for Eurasian Reed Warblers and Great Reed Warblers ($O_{jk} = 0.35$), but still higher than expected by chance, reflecting greater sampling uncertainty for the consumer with the smallest sample size. Nevertheless, there was significantly greater niche overlap than expected by chance in

Table 1. The most frequently detected prey taxa from faecal samples of Cetti's Warbler, Great Reed Warbler and Eurasian Reed Warbler, ranked from #1 to 43. Samples from a small number of recaptured individuals ($n = 8$) were included in the % FOO calculations but are omitted from later statistical analyses. A total of 43 taxa are displayed as this value includes at least the top 20 prey items for each warbler species, where % FOO is equivalent to at least two samples testing positive for the taxa in Great Reed Warbler and Cetti's Warbler (the species with the smallest sample size; $n = 26$) and six samples for the Eurasian Reed Warbler ($n = 137$). Taxa with the same frequency of occurrence (% FOO) in each warbler's diet should be considered as equal in rank. Cells coloured green indicate taxa ranked #43 or higher in more than one list.

Cetti's Warbler ($n = 26$)			Great Reed Warbler ($n = 26$)		Eurasian Reed Warbler ($n = 137$)	
Rank	Prey taxa	% FOO	Prey taxa	% FOO	Prey taxa	% FOO
1.	Psoctera sp. ^a	26.9%	<i>Ruspolia nitidula</i> (Orthoptera: Tettigoniidae)	26.9%	<i>Chironomus plumosus</i> (Diptera: Chironomidae)	18.2%
2.	<i>Lepyronia coleoptrata</i> (Hemiptera: Aphrophoridae)	23.1%	<i>Chironomus sp.</i>^a (Diptera: Chironomidae)	23.1%	<i>Ochlerotatus caspius</i> (Diptera: Culicidae)	16.1%
3.	<i>Chironomus riparius</i> (Diptera: Chironomidae)	19.2%	<i>Clubiona phragmitis</i> (Araneae: Clubionidae)	15.4%	<i>Chironomus sp.</i> (Diptera: Chironomidae)	14.6%
4.	<i>Philodromus cespitum</i> (Araneae: Philodromidae)	19.2%	<i>Simyra nervosa</i> (Lepidoptera: Noctuidae)	15.4%	<i>Clubiona phragmitis</i> (Araneae: Clubionidae)	14.6%
5.	<i>Apis mellifera</i> (Hymenoptera: Apidae)	15.4%	<i>Episyrphus sp.</i> ^a (Diptera: Syrphidae)	11.5%	<i>Chironomus nudatarsis</i> (Diptera: Chironomidae)	12.4%
6.	<i>Chironomus sp.</i>^a (Diptera: Chironomidae)	15.4%	<i>Chironomus nudatarsis</i> (Diptera: Chironomidae)	11.5%	<i>Cryptochironomus obreptans</i> (Diptera: Chironomidae)	8.8%
7.	<i>Neophilaenus lineatus</i> (Hemiptera: Aphrophoridae)	15.4%	<i>Clytie illunaris</i> (Lepidoptera: Erebiidae)	11.5%	<i>Cricotopus sylvestris</i> (Diptera: Chironomidae)	8.8%
8.	<i>Polypogon plumigeralis</i> (Lepidoptera: Erebiidae)	15.4%	<i>Ichneumonidae sp.</i> ^a (Hymenoptera)	11.5%	<i>Ochlerotatus detritus</i> (Diptera: Culicidae)	7.3%
9.	<i>Amphorophora rubi</i> (Hemiptera: Aphididae)	11.5%	<i>Lacanobia oleracea</i> (Lepidoptera: Noctuidae)	11.5%	<i>Diplazon laetatorius</i> (Hymenoptera: Ichneumonidae)	5.8%
10.	<i>Axylla putris</i> (Lepidoptera: Noctuidae)	11.5%	<i>Sympetrum fonscolombii</i> (Odonata: Libellulidae)	11.5%	<i>Sesamia nonagrioides</i> (Lepidoptera: Noctuidae)	5.8%
11.	<i>Dasysyrphus albostratus</i> (Diptera: Syrphidae)	11.5%	<i>Chironomus plumosus</i> (Diptera: Chironomidae)	7.7%	<i>Orthocladinae sp.</i> (Diptera: Chironomidae)	5.8%
12.	<i>Idaea dimidiata</i> (Lepidoptera: Geometridae)	11.5%	<i>Cryptochironomus obreptans</i> (Diptera: Chironomidae)	7.7%	<i>Cricotopus bicinctus</i> (Diptera: Chironomidae)	5.8%
13.	<i>Plutella xylostella</i> (Lepidoptera: Plutellidae)	11.5%	<i>Diplazon laetatorius</i> (Hymenoptera: Ichneumonidae)	7.7%	<i>Chironomus riparius</i> (Diptera: Chironomidae)	5.8%
14.	<i>Pollenia leclercqiana</i> (Diptera: Calliphoridae)	11.5%	<i>Sesamia nonagrioides</i> (Lepidoptera: Noctuidae)	7.7%	<i>Aphidius transcaspicus</i> (Hymenoptera: Braconidae)	5.1%
15.	<i>Cecidomyiidae sp.</i> ^a	11.5%	<i>Chironomus curabilis</i> (Diptera: Chironomidae)	7.7%	<i>Aphis nerii</i> (Hemiptera: Aphididae)	5.1%
16.	<i>Chironomus transvaalensis</i> (Diptera: Chironomidae)	11.5%	<i>Hyalopterus pruni</i> (Hemiptera: Aphididae)	7.7%	<i>Orthocladus fuscimanus</i> (Diptera: Chironomidae)	5.1%
17.	<i>Clubiona terrestris</i> (Araneae: Clubionidae)	11.5%	<i>Anacridium aegyptium</i> (Orthoptera: Acrididae)	7.7%	<i>Chironomus curabilis</i> (Diptera: Chironomidae)	4.4%
18.	<i>Cricotopus sylvestris</i> (Diptera: Chironomidae)	11.5%	<i>Anoplius caviventris</i> (Hymenoptera: Pompilidae)	7.7%	<i>Cecidomyiidae sp.</i> ^a (Diptera)	4.4%
19.	<i>Diplazon laetatorius</i> (Hymenoptera: Ichneumonidae)	11.5%	<i>Coreus marginatus</i> (Hemiptera: Coreidae)	7.7%	<i>Eupeodes sp.</i> ^a (Diptera: Syrphidae)	4.4%
20.	<i>Lysiphlebus testaceipes</i> (Hymenoptera: Braconidae)	11.5%	<i>Enicospilus sp.</i> ^a (Hymenoptera: Ichneumonidae)	7.7%	<i>Philodromus cespitum</i> (Araneae: Philodromidae)	4.4%
21.	<i>Stilbus testaceus</i> (Coleoptera: Philacridae)	11.5%	<i>Orthetrum albistylum</i> (Odonata: Libellulidae)	7.7%	<i>Hyalopterus sp.</i> ^a (Hemiptera: Aphididae)	4.4%
22.	<i>Abrostola triplasia</i> (Lepidoptera: Noctuidae)	7.7%	<i>Melanips sp.</i> ^a (Hymenoptera: Figitidae)	7.7%	<i>Salticus zebraneus</i> (Araneae: Salticidae)	4.4%

(continued)

Table 1. (continued)

Cetti's Warbler (<i>n</i> = 26)			Great Reed Warbler (<i>n</i> = 26)		Eurasian Reed Warbler (<i>n</i> = 137)	
Rank	Prey taxa	% FOO	Prey taxa	% FOO	Prey taxa	% FOO
23.	<i>Armadillidium vulgare</i> (Isopoda: Armadillidiidae)	7.7%	<i>Autographa gamma</i> (Lepidoptera: Noctuidae)	7.7%	<i>Corimalia tamarisci</i> (Coleoptera: Brentidae)	4.4%
24.	<i>Capitophorus pakansus</i> (Hemiptera: Aphididae)	7.7%	<i>Nacerdes melanura</i> (Coleoptera: Oedemeridae)	7.7%	<i>Praon abjectum</i> (Hymenoptera: Braconidae)	4.4%
25.	<i>Clubiona phragmitis</i> (Araneae: Clubionidae)	7.7%	<i>Orthocladus oblidens</i> (Diptera: Chironomidae)	7.7%		
26.	<i>Coenosia</i> sp. ^a (Diptera: Muscidae)	7.7%	<i>Rhaphigaster nebulosa</i> (Hemiptera: Pentatomidae)	7.7%		
27.	<i>Episyrphus</i> sp. ^a (Diptera: Syrphidae)	7.7%				
28.	<i>Eupithecia ultimaria</i> (Lepidoptera: Geometridae)	7.7%				
29.	<i>Fannia canicularis</i> (Diptera: Fanniidae)	7.7%				
30.	<i>Heterotoma planicornis</i> (Hemiptera: Miridae)	7.7%				
31.	<i>Lachesilla pedicularia</i> (Psocoptera: Stenopsocidae)	7.7%				
32.	<i>Lenisa geminipuncta</i> (Lepidoptera: Noctuidae)	7.7%				
33.	<i>Meteorus pendulus</i> (Hymenoptera: Braconidae)	7.7%				
34.	<i>Orthocladinae</i> sp. (Diptera: Chironomidae)	7.7%				
35.	<i>Otala punctata</i> (Gastropoda: Helicidae)	7.7%				
36.	<i>Paradromius longiceps</i> (Coleoptera: Carabidae)	7.7%				
37.	<i>Peribatodes rhomboidaria</i> (Lepidoptera: Geometridae)	7.7%				
38.	<i>Philodromus ruficapillus</i> (Araneae: Philodromidae)	7.7%				
39.	<i>Phlogophora meticulosa</i> (Lepidoptera: Noctuidae)	7.7%				
40.	<i>Polypedilum cultellatum</i> ^b (Diptera: Chironomidae)	7.7%				
41.	<i>Pseudolycoriella compacta</i> (Diptera: Sciaridae)	7.7%				
42.	<i>Psychodidae</i> sp.^a (Diptera)	7.7%				
43.	<i>Sesamia nonagrioides</i> (Lepidoptera: Noctuidae)	7.7%				

^aUnidentified species. ^bBold type indicates taxa with an aquatic or semi-aquatic origin.

85% of bootstrap replicates, indicating that uneven sampling effort did not alter the overall overlap inferred.

In contrast, significant dietary partitioning was detected between Great Reed Warblers and Cetti's Warblers ($O_{jk} = 0.23$, $P = 0.001$). Pianka's overlap was significantly lower than expected under the RA3 null model, and this finding was robust to

bootstrap resampling, with niche partitioning detected in 79% of replicates.

We detected dietary overlap between Eurasian Reed Warblers and Cetti's Warblers, with a moderate Pianka overlap value ($O_{jk} = 0.4$, $P = 0.04$). Only 31% of bootstrap replicates showed significantly elevated overlap from that expected from the null model, however, indicating that this result

Table 2. Frequency of occurrence (% FOO) of major invertebrate orders detected in Cetti's Warbler, Great Reed Warbler and Eurasian Reed Warbler faecal samples. Values in parentheses represent the total number of species (species richness) detected within each consumer category for each invertebrate order. Samples from a small number of recaptured individuals ($n = 8$) are included in the % FOO calculations but are omitted from later statistical analyses. Cells are colour coded by frequency of occurrence (darker blue tone = greater % FOO value).

Order	All consumers ($n = 189$)	Cetti's Warbler ($n = 26$)	Great Reed Warbler ($n = 26$)	Eurasian Reed Warbler ($n = 137$)
Araneae	38.62 (33)	61.54 (19)	19.23 (2)	37.96 (23)
Coleoptera	21.16 (26)	42.31 (13)	23.08 (5)	16.79 (14)
Decapoda	1.06 (1)	–	3.85 (1)	0.79 (1)
Diptera	74.60 (90)	80.77 (36)	53.85 (21)	77.4 (72)
Gastropoda	3.70 (7)	23.08 (7)	3.85 (1)	–
Hemiptera	30.69 (33)	57.69 (19)	42.31 (10)	23.36 (14)
Hymenoptera	47.61 (53)	61.54 (16)	61.54 (12)	42.35 (39)
Isopoda	2.12 (1)	7.69 (1)	–	1.46 (1)
Julida	0.53 (1)	3.85 (1)	–	–
Lepidoptera	54.50 (73)	80.77 (32)	53.85 (16)	49.64 (44)
Mecoptera	1.06 (1)	–	–	1.46 (1)
Neuroptera	5.29 (4)	11.54 (3)	–	5.11 (4)
Odonata	3.70 (3)	–	15.38 (2)	2.19 (2)
Opiliones	0.53 (1)	3.85 (1)	–	–
Orthoptera	7.41 (3)	3.85 (1)	34.62 (3)	2.92 (2)
Psocoptera	7.41 (2)	30.77 (2)	–	4.38 (2)
Thysanoptera	0.53 (1)	3.85 (1)	–	–
Trichoptera	2.65 (2)	3.85 (1)	3.85 (1)	2.19 (2)
Aquatic Species	71.43	61.54	68.23	73.72
Terrestrial Species	89.42	100	92.31	86.86

was sensitive to sampling variation and should be interpreted with caution.

A summary of the bootstrap resampling diagnostics is available in Data S10.

Dietary niche breadth

Dietary niche breadth, standardized at 50% coverage, differed significantly among the three consumers, as indicated by non-overlapping CIs

(Fig. 3). Cetti's Warblers (106.3) had the greatest core dietary niche breadth ($q = 2$) of the three species, closely followed by Eurasian Reed Warblers (75.9), with Great Reed Warblers (45.8) having a substantially narrower diet in comparison.

Predictors of diet composition

Diet composition differed between consumer species ($P = 0.001$) and sites ($P = 0.001$, Table 4), with dietary differences between species illustrated by the NMDS plot (Fig. 4). Results of the multivariate analyses were consistent across prey taxonomic ranks (Data S12). Univariate tests revealed a significant difference in the frequency of just one species, *Ochlerotatus caspius*, in the diets of the three warblers (LRT = 12.8, $P = 0.042$), while several prey species differed in occurrence among the diets of warblers captured at different sites.

Prey size and selection of aquatic prey

Mean prey size (body length) differed significantly between warbler species (likelihood ratio test: $F = 35.79$, $df = 2$, $P < 0.0001$, Table 5a). Great Reed Warbler samples contained prey species with a greater mean body length (16.1 ± 1.8 mm) compared to either Eurasian Reed Warblers (7.7 ± 1.8 mm; $z = 5.71$, $P = 0.001$) or Cetti's Warblers (7.9 ± 0.59 mm; $z = 4.41$, $P = 0.001$). However, prey species size did not differ significantly between Eurasian Reed Warblers and Cetti's Warblers ($z = 1.15$, $P = 0.48$, Table 5b). Site was a marginally significant predictor of body length ($F = 2.77$, $df = 3$, $P = 0.043$).

The proportion of aquatic prey differed between warbler species ($F = 8.53$, $df = 2$, $P = 0.00029$, Table 5a; Fig. 5). Pairwise comparisons of estimated marginal means (Table 5b) showed that Eurasian Reed Warbler diet samples had a higher proportion of aquatic prey (37% of prey taxa) than Cetti's Warbler samples (17%; $z = 3.1$, $P = 0.0054$), but neither differed significantly from Great Reed Warbler (29%). Site was also a significant predictor of aquatic prey in diet samples ($F = 9.35$, $df = 3$, $P < 0.0001$).

DISCUSSION

Our study is the first to use DNA metabarcoding to study interspecific dietary patterns in reedbed passerines, a community at risk of being adversely

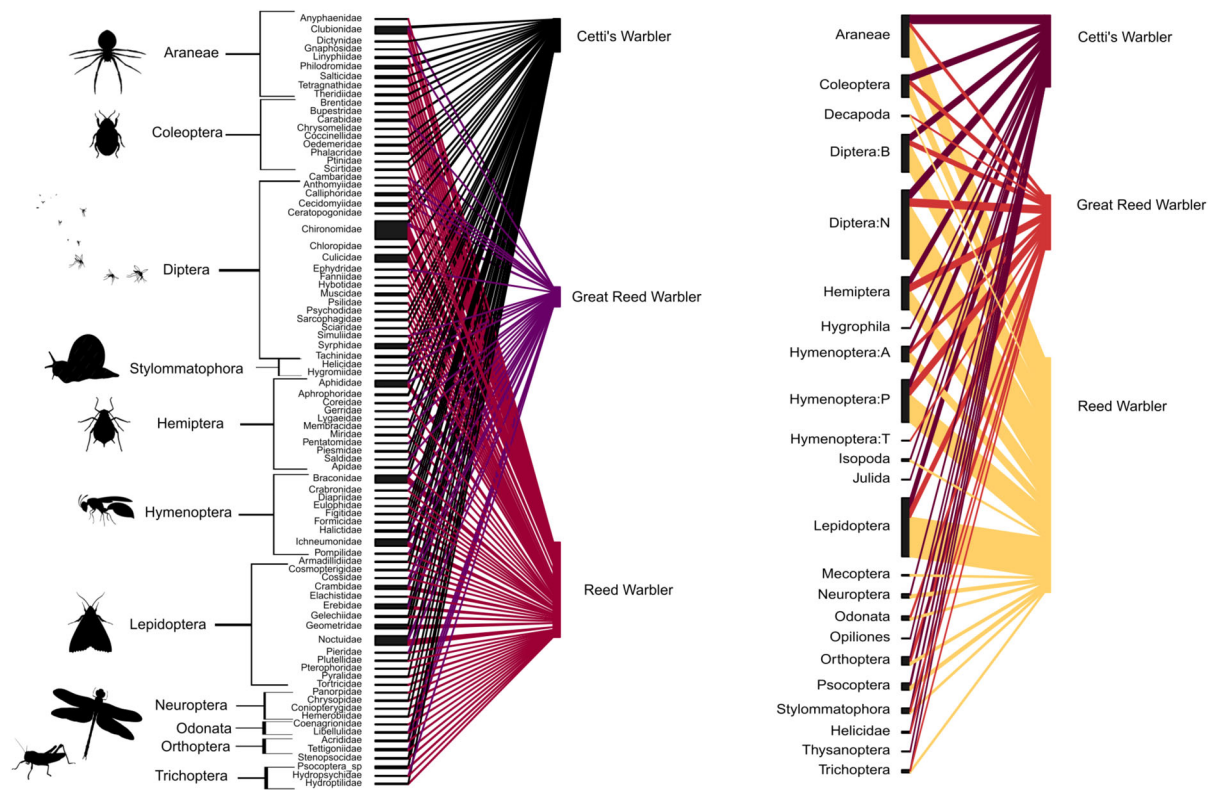


Figure 2. Bipartite network plot presenting the dietary niches of the three warbler species. The width of the connecting arrows each corresponds with the frequency of occurrence of the prey (i) families (left) and (ii) orders (right) detected across the faecal samples collected for each focal bird species. In the order-level plot, Diptera B refers to Diptera within the sub-order Brachycera, whereas Diptera N refers to Diptera in the sub-order Nematocera. Hymenoptera A refers to sub-grouping Aculeata and Hymenoptera P refers to Parasitica, while Hymenoptera T refers to the superfamily Tenthredinoidea (sawflies).

impacted by climate change and insect prey declines throughout their range. As predicted, the three warbler species exhibited clear differences in prey resource use and core niche breadth, and partitioned prey via several mechanisms: prey taxonomic group, prey size and the proportion of aquatic prey consumed. Great Reed Warbler diet tended towards larger prey, and the narrowest core dietary niche; Eurasian Reed Warblers consumed the greatest proportion of aquatic prey, while Cetti's Warblers generally showed the broadest core dietary niche, including the greatest proportion of terrestrial prey.

We detected differences in diet composition, the proportion of aquatic prey in diets, and to a lesser extent prey size selection between all warblers sampled at different study sites. While explanatory variables relating to capture locality were not explicitly assessed in this study, these findings may be partially due to dietary plasticity

in response to different compositions of prey communities in different locations, or differences in microhabitat use among localities of varied habitat structure and composition. Arthropod abundance and diversity are known to differ among the sites studied (Davies 2020), which could influence the observed patterns of prey selection and diet composition among warblers in different localities. Moreover, significant prey partitioning was observed between Great Reed Warblers and Cetti's Warblers, but there were significant levels of dietary overlap between Eurasian Reed Warblers and Great Reed Warblers, and to a lesser degree between Eurasian Reed Warblers and Cetti's Warblers. For the latter pair, bootstrap resampling indicated that overlap estimates were sensitive to sampling variation and were therefore less robust than for the other species pairs.

Although we could not directly measure competitive pressure with this DNA-based approach,

Table 3. Pianka's measure of dietary overlap (O_{jk}) between pairs of warbler species. Expected overlap values (mean and variance (latter in parentheses)) of the simulated index predicted by the null model, and the standardized effect size (SES) are reported for each pairwise analysis below the observed value. Negative SES values and an orange shade indicate a lower observed overlap value than predicted by the null model, whereas positive SES values and a green shade indicate a higher observed overlap value than predicted by the null model.

		Eurasian Reed Warbler	Cetti's Warbler
Great Reed Warbler	Observed	0.45**	0.23**
	Expected	0.27 (var = 0.0022)	0.39 (var = 0.0018)
	SES	3.95	-3.68
Eurasian Reed Warbler	Observed		0.40*
	Expected		0.33 (var = 0.0015)
	SES		1.82

Asterisks denote significantly higher or lower overlap than predicted from the null model (* $P < 0.05$; ** $P < 0.01$).

dietary partitioning is likely to be one of the main mechanisms permitting coexistence during heightened periods of resource competition in songbirds, such as during the breeding season. The moderately high degree of diet similarity between two of the three species pairs can be explained by each species consuming common, frequently encountered prey taxa.

Dietary overlap and optimal foraging

We hypothesized that warbler pairs would partition prey and show low levels of dietary overlap. Optimal foraging theory predicts that consumers should specialize on a few highly profitable and abundant prey types to maximize returns per unit of energy expenditure and expand their diet to a wider range of prey types when these 'preferred' prey become rare (MacArthur & Pianka 1966, Pulliam 1985, Perry & Pianka 1997, Martínez-Curci *et al.* 2015). During prey-limited periods, consumer diets broaden and converge on the remaining prey available, with high dietary overlap then driving stronger competition between species as resources become depleted (Clare *et al.* 2014, Salinas-Ramos *et al.* 2015, Cutting *et al.* 2016). It is unlikely that the levels of overlap observed in our study reflect severe food shortage; however, because warbler diet composition showed subtle but significant differences between species, suggesting that consumers were not restricted in their prey selectivity, and still partitioned prey as predicted under optimal foraging theory.

Where dietary overlap was significant, Pianka values did not exceed 0.5, suggesting 'moderate' overlap (Novakowski *et al.* 2008) consistent with the widespread sharing of abundant prey that were available across multiple sites. When prey availability peaks, the diets of birds with different foraging strategies may converge if each species

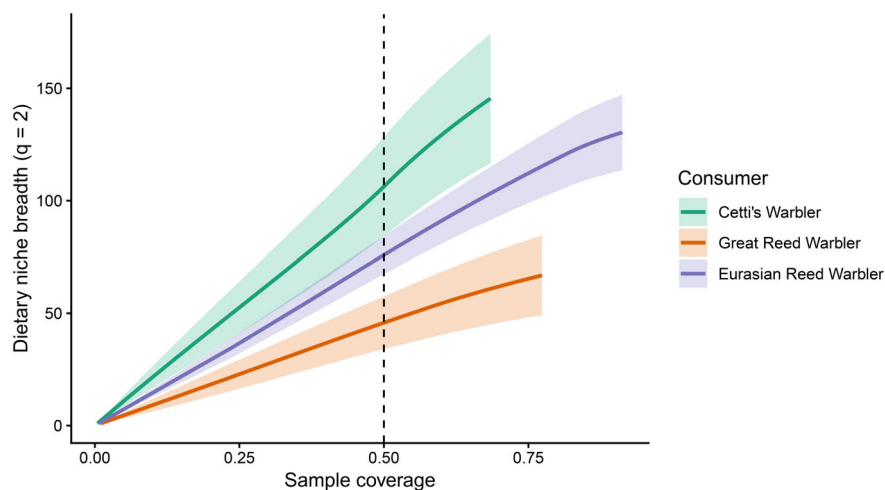


Figure 3. Coverage-based rarefaction and extrapolation curves for Simpson-based dietary niche breadth ($q = 2$) for each of the three warbler consumers. The $q = 2$ value reflects the effective number of dominant prey in the diet (core dietary breadth). The dotted, vertical line indicates dietary niche breadth at the selected target sample coverage (50%). Shaded areas around the curves represent 95% confidence intervals around the estimates.

Table 4. Test statistics for variables included in the *manyglm* analysis investigating dietary differences between warbler species and study sites. Individual prey species identified in the univariate tests that varied significantly in warbler diets in line with one or more of the modelled variables are also listed and the level of statistical significance is reported.

Tested variable	Residual degrees of freedom	Df. diff	Deviance	P value
Multivariate test				
Intercept	180			
Species	178	2	539.5	0.001***
Site	175	3	1530.7	0.001***
Pairwise tests				
Species pair			Deviance	P value
Cetti's Warbler			608.3	0.001***
–Eurasian Reed Warbler				
Great Reed Warbler			400.5	0.001***
–Eurasian Reed Warbler				
Cetti's Warbler			370.9	0.001***
–Eurasian Reed Warbler				
Univariate tests				
Tested variable and prey taxon			LRT	P value
Species: <i>Ochlerotatus caspius</i>			12.80	0.042*
Site: <i>Orthocladia fuscimanus</i>			20.16	0.009**
Site: <i>Leucania obsoleta</i>			18.32	0.015*
Site: <i>Chironomus curabilis</i>			16.06	0.052
Site: <i>Aphidius transcaspicus</i>			17.13	0.023*
Site: <i>Cricotopus bicinctus</i>			27.43	0.002**
Site: <i>Philodromus cespitum</i>			16.94	0.026*
Site: <i>Chironomus nuditarsis</i>			21.76	0.01**
Site: <i>Ochlerotatus caspius</i>			17.89	0.017*
Site: <i>Chironomus plumosus</i>			16.89	0.026

Significance codes: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

opportunistically targets the same abundant prey taxa (Wiens & Rotenberry 1979, Rotenberry 1980, Rosenberg *et al.* 1982). Similar levels of overlap between Neotropical migratory songbird nestlings were described in an earlier metabarcoding study (Trevelline *et al.* 2018b), consistent with dietary opportunism permitted by an abundance of riparian insect prey during peak periods of insect availability. The high prevalence of locally abundant prey groups in the diet of all three warblers in our study, therefore, probably masks a degree of species-specific prey selectivity expected under dietary partitioning. Each warbler species specializes on a subset of profitable prey, but may expand this to consume locally abundant, non-limiting prey that, due to their high local

abundance, can be shared by multiple consumers. These prey are energetically 'profitable' under optimal foraging, as it is less energetically costly to locate and capture abundant prey than it is to search for rarer prey.

Many of the prey taxa consumed by more than one warbler species were highly mobile arthropod species that can readily disperse between microhabitats. Thus, even though each warbler species may forage in different microhabitats within the reedbed, these same prey species are likely to be encountered by all three warbler species (Wilson 2010, Kent & Sherry 2020). For example, chironomids were detected at high frequency in warbler faecal samples in the present study and were regularly captured on sticky traps placed in different microhabitats at the breeding sites over the same period (Davies 2020). Due to their wide emergence time-window, and often very high local abundance during emergence events, chironomids are generally widely available and form an important component of the diet of many wetland passerines (Dyrce & Flinks 2000, López-Iborra *et al.* 2005, King *et al.* 2015).

The greatest reported dietary overlap was between Eurasian Reed Warblers and Great Reed Warblers. Despite some horizontal and vertical separation in habitat selection (Dyrce 1981, Leisler 1981), Eurasian Reed Warblers and Great Reed Warblers nest in reedbeds in both terrestrial and open-water zones (Cramp & Brooks 1992). During the breeding season, habitat segregation between these two species is limited, which may have resulted in converging diets from encountering similar, abundant prey (Trevelline *et al.* 2018b). We did, however, detect some significant differences in diet composition and prey size selection, suggesting that both species maintain a distinct dietary niche.

Dietary overlap between Cetti's Warblers and Great Reed Warblers was significantly lower than expected by the null model, suggesting a higher degree of dietary partitioning between these two species, perhaps due to stronger pairwise divergences in foraging habitat, morphology and life-history traits (Cramp & Brooks 1992, Araújo *et al.* 2016).

Cetti's Warblers tend to nest in terrestrial habitats such as low, dense vegetation and scrub. Their foraging sites may be near water and include some reedbed areas (Molina *et al.* 1998, Araújo *et al.* 2016), but they never include open-water zones as is the case for the Great Reed Warbler (Harvey 1977, Bibby & Thomas 1984,

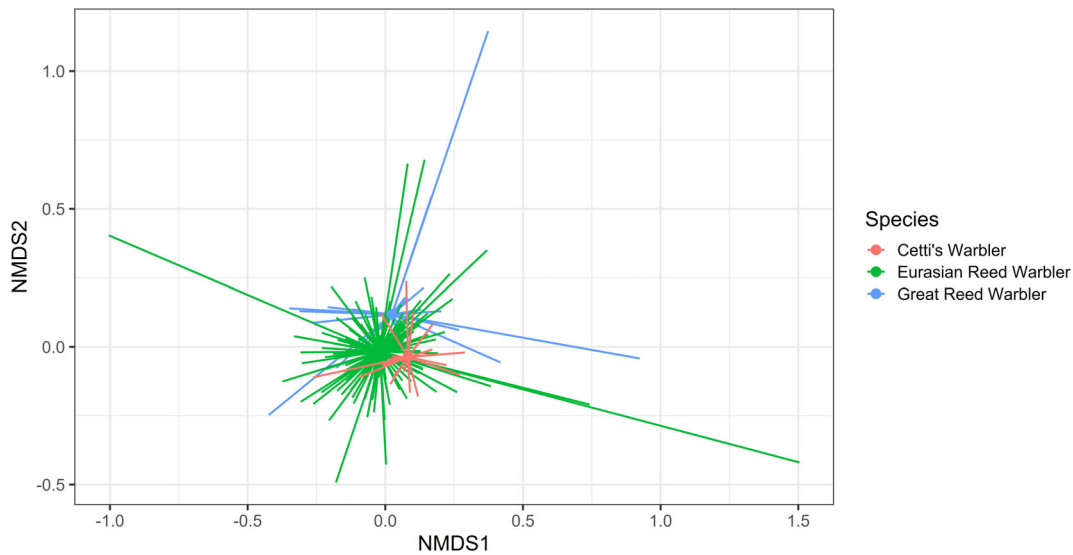


Figure 4. Non-metric multi-dimensional scaling (NMDS) plots of the invertebrate prey species detected in warbler diet samples collected in Catalonia grouped by warbler species. Stress = 0.08. Stress measures how well the distances between points in the 2D ordination plot reflect the actual dissimilarities (distances) in the original high-dimensional data matrix. A lower stress value (ideally < 0.2) indicates that the reduced space accurately preserves the relative ranks of distances from the original data.

Table 5a. Test statistics for the two GLMs investigating differences in prey size and selection of aquatic prey by reedbed warblers.

Model response variable	Tested predictor variable	Residual degrees of freedom	df	F	P value
Prey body length	Species	178	2	35.79	< 0.0001***
	Site	175	3	2.77	0.043*
	Res. std error	F statistic	df	Adjusted R-squared	P value
	4.62	16.81	5 and 175	0.3051	< 0.0001***
	Tested predictor variable	Residual degrees of freedom	df	F	P value
Proportion of aquatic prey species	Species	178	2	8.53	0.000291***
	Site	175	3	9.35	< 0.0001***
	Res. std error	F statistic	df	Adjusted R-squared	P value
	0.52	8.66	5 and 175	0.176	< 0.0001***

Significance codes: **P* < 0.05; ****P* < 0.001.

Balança & Schaub 2005). These habitat associations, alongside the reduced dietary similarity with Great Reed Warbler, could explain why Cetti's Warbler diets comprised a smaller proportion of aquatic species than the other warblers.

Niche breadth

Great Reed Warblers had the narrowest diet breadth of the three species, followed by Eurasian

Reed Warblers and Cetti's Warblers. The narrower core foraging niche breadth of Great Reed Warblers suggests a more specialized diet in more aquatic habitat. Interestingly, this contrasts with the theory that resident species are more selective than migrants, or that migrants are forced to expand their diets to consume less profitable prey (Poulin & Lefebvre 1996). Differences in body size, morphology and habitat use between these species may therefore play a larger role in

Table 5b. Pairwise comparisons of estimated marginal means (least-squares means) with a 'Tukey' adjustment (for multiple comparisons) for GLMs investigating differences in prey size and selection of aquatic prey by reedbed warblers.

Model predictors	Pairwise tests	z-value	P value
<i>Prey body length</i> Species	Cetti's Warbler–Eurasian Reed Warbler	1.15	0.48
	Great Reed Warbler–Cetti's Warbler	4.41	0.001***
	Great Reed Warbler–Eurasian Reed Warbler	5.71	0.001***
<i>Proportion of aquatic prey</i> Species	Cetti's Warbler–Great Reed Warbler	−0.869	0.66
	Cetti's Warbler–Eurasian Reed Warbler	−3.104	0.0054**
	Great Reed Warbler–Eurasian Reed Warbler	−1.92	0.32

Significance codes: ** $P < 0.01$; *** $P < 0.001$.

determining the degree of dietary specialization and plasticity. For example, Cetti's Warblers use a variety of densely vegetated terrestrial and semi-aquatic habitats during the breeding season, where a diverse range of arthropods may be encountered. The broader core foraging niche observed in Cetti's Warblers suggests a more generalized insectivorous strategy, consistent with its wider and more terrestrial habitat use, making it able to exploit a wider range of abundant prey species and show greater foraging flexibility.

Simpson-based taxonomic diversity accumulation curves (focusing on the dominant or 'core' prey present in the diet) stabilized slowly, suggesting that fully characterizing dietary niche breadth in these systems may require substantially larger sampling effort. Nonetheless, our study allowed us to identify differences in relative core dietary niche breadth among warbler species.

Prey selection

Morphological differences in warbler body and bill size, and different dietary requirements between warbler species, lead to differential prey-size selection and reduced foraging competition (Marchetti *et al.* 1996). Earlier studies suggest that Eurasian

Reed Warblers and Great Reed Warblers take prey of different sizes (Bussmann 1979, Leisler 1991, Schulze-Hagen 1991, Cramp & Brooks 1992). Likewise, in the present study, the three warbler species deviated significantly in their selection of prey size classes. Aligning with a study of diet among seven warbler species in Western France (Bibby & Green 1983), in the present study, the largest prey species were consumed by the largest warbler species. Great Reed Warbler diet included some very large prey species not consumed by the other two warblers: Egyptian Locust *Anacridium aegyptium*, White-tailed Skimmer *Orthetrum albigyllum*, Elephant Hawkmoth *Deilephila elpenor* and Hornet Clearwing *Sesia apiformis*.

For smaller-bodied warbler species, the energetic cost of capture and handling time needed to consume large prey may exceed the calorific profit (Turner 1982, Pulliam 1985). If, however, the larger size of the Great Reed Warbler brings both greater calorific demand and an increased ability to handle large prey, then selecting larger prey would be more efficient (Turner 1982, Robinson & Wilson 1998). This was illustrated by an earlier study where Great Reed Warblers brought only the largest female orb-web spiders to their chicks, whereas Eurasian Reed Warblers in the same location provisioned their nestlings with smaller spiders of the same species (Leisler & Schulze-Hagen 2011). Similarly, in Southern France, the Great Reed Warbler specialized on larger prey relative to its body and bill size, compared to the Eurasian Reed Warbler and Moustached Warbler *A. melanopogon* (Bussmann 1979). As they can only hold a few prey items in their bill at a time, Great Reed Warblers in Bussmann's (1979) study brought a smaller number of prey items comprising a greater total mass per feed to their nestlings and provisioned at a lower rate, whereas the other species captured smaller prey in greater numbers, with a higher feeding rate, resulting in roughly equal total masses of food provisioned per hour across all three species. Different specializations in foraging and prey size thereby allow warbler species to coexist with only minimal direct competition for food.

As well as morphology and body size, prey size partitioning between similar species can be a result of ecological separation, as observed in aerial insectivores (hirundines and swifts) feeding at different air-space heights (Orłowski & Karg 2013), and sympatric bats foraging in distinct habitats

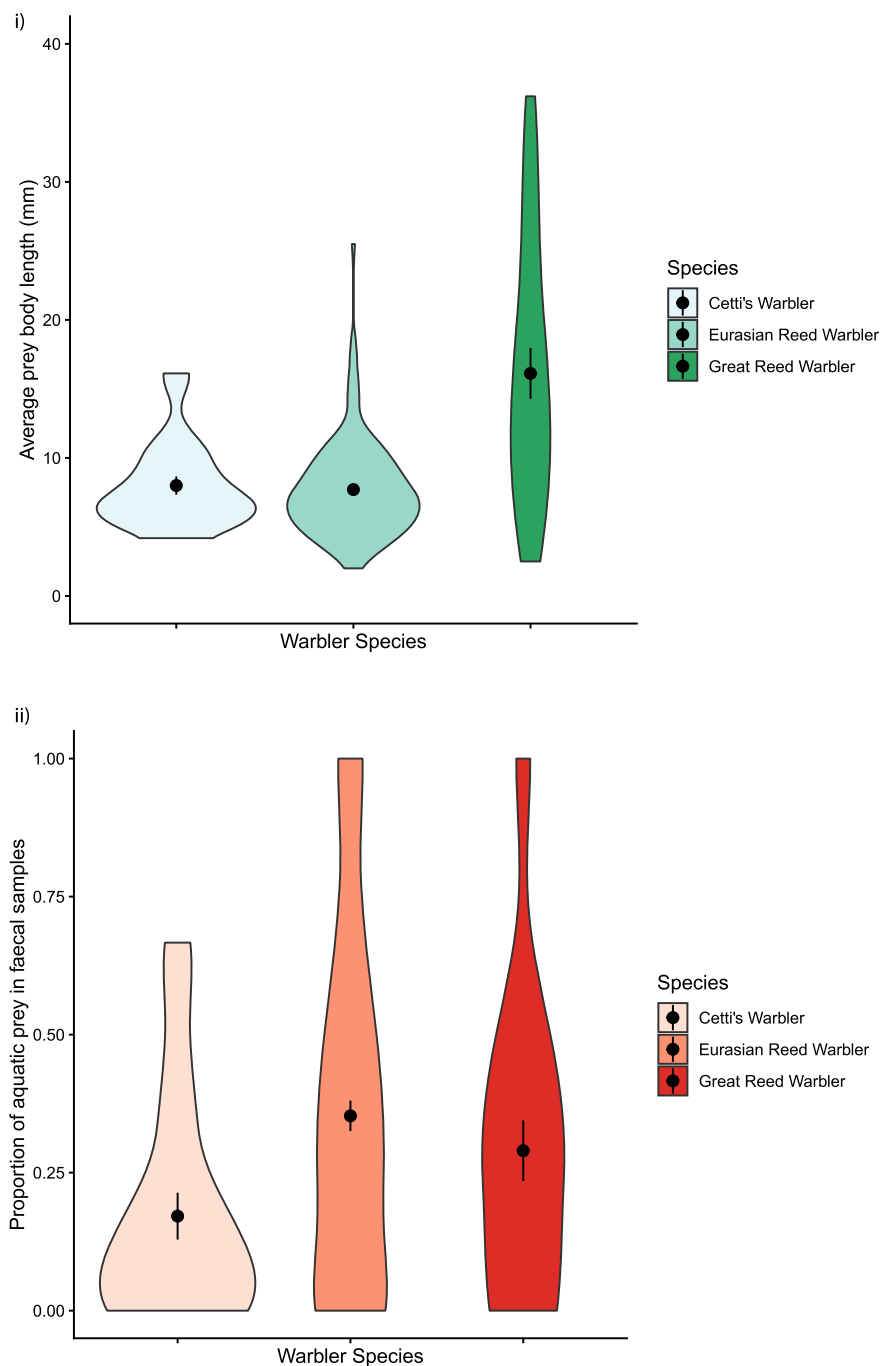


Figure 5. Violin plots of: (i) mean body size (length in mm) of prey taxa in warbler faecal samples and (ii) the proportion of aquatic prey taxa in warbler faecal samples among the three focal warbler species.

(Vesterinen *et al.* 2018). Size segregation can also occur due to one competitor suppressing a second competitor's access to specific types of prey. The Great Reed Warbler is known to exclude the Eurasian Reed Warbler from profitable nesting sites in

areas where they coexist (Dyrce 1981, Hoi *et al.* 1991, Honza *et al.* 1999, Schaefer *et al.* 2006). This may lead to Eurasian Reed Warblers encountering smaller prey than Great Reed Warblers, although the energetic cost of selecting

the largest prey available may also be too high for the former.

The frequent occurrence of emerging aquatic insects in the diets of all three study species aligns with the wider literature, although Eurasian Reed Warblers showed a particular affinity for aquatic prey. A growing number of studies document the value of emerging aquatic prey (or aquatic subsidies) for terrestrial consumers such as songbirds (Trevelline *et al.* 2016, Twining *et al.* 2018, Lewis-Phillips *et al.* 2020, Berzins *et al.* 2021). Aquatic prey are rich in omega-3 long-chain polyunsaturated fatty acids (*n*-3 LC-PUFAs), important for development and cognition in birds and other terrestrial animals (Hixon *et al.* 2015, Martin-Creuzburg *et al.* 2017, Twining *et al.* 2019, 2021, Berzins *et al.* 2021). These compounds are lacking in terrestrial prey, so insectivores in riparian habitats predominantly acquire LC-PUFAs from aquatic insects (Lam *et al.* 2013, Martin-Creuzburg *et al.* 2017, Twining *et al.* 2019, Kowarik *et al.* 2021, Fehlinger *et al.* 2022). Warming summer temperatures in the Mediterranean are likely to exacerbate the loss of permanently wet aquatic habitats (Jiménez *et al.* 2018), which has implications for future aquatic prey availability for insectivorous birds.

Warbler age may also play a role in determining the relative importance of aquatic groups to the diet. Davies *et al.* (2022) found that aquatic prey were taken more frequently by juvenile (post-fledging) Eurasian Reed Warblers in the UK compared to adults, and similar age-related dietary variation may be present in our study sites.

Study limitations and recommendations

The proportion of samples from each of the focal warbler species matched the relative proportions of captures in mist-nets (inferred from ICO ringing data for each site in 2018). Eurasian Reed Warblers dominated the catches numerically, suggesting high population density, whereas Great Reed Warblers and Cetti's Warblers were captured less frequently. The potential impacts of sample unevenness were considered in our analysis of dietary overlap and niche breadth. Collection of a larger number of samples at each of the sites over the summer and over multiple years would allow more accurate characterization of diets and reveal trends over time and space.

Our prey size analysis used adult (imago) reference body sizes, as the life stages of prey consumed by the warblers sampled in the present study were not possible to elucidate. As we expect at least a proportion of Lepidoptera consumed to be larvae, error may be introduced when using imago reference values, causing an over- or underestimate of average prey body size per diet sample. Given that most of the prey available to warblers are likely to be emerged and winged (flying) imagoes, particularly for very diverse taxa such as Diptera, we expect this error to be relatively small when all taxa are considered together. Size differences between diverse prey taxa are generally greater than differences within species (even among life stages), and small deviations in prey body sizes are very unlikely to account for the clear and consistent differences in prey size selection identified between Great Reed Warblers and the two other warbler species.

Dietary overlap may change over time, due to seasonal and between-year fluctuations in prey composition and abundance (Wiens 1977, McMartin *et al.* 2009). Weather fluctuations can also alter microhabitat selection and feeding intensity by birds by directly altering the distribution, abundance or activity of arthropods (Petit *et al.* 1990). We did not have a large enough sample size of Cetti's Warblers and Great Reed Warblers to assess temporal changes in pairwise dietary overlap between species, but as shifts in local climate, stochastic weather events and seasonality all influence prey availability and transform the foraging habitats available to birds (e.g. re-wetting and drying of aquatic zones, reedbed die-back), we expect an influence on prey availability and dietary composition.

CONCLUSION

The three study species partitioned their prey via several mechanisms, particularly by (i) taxonomic group, (ii) prey size and (iii) proportion of aquatic prey consumed. The latter two mechanisms were particularly important in distinguishing the diet of Great Reed Warblers (larger prey body size) and Cetti's Warblers (terrestrial prey).

In the coming decades, climate change may drive stronger resource partitioning in sympatric warbler species in European wetlands. With warmer conditions and increasing reedbed drought during the summer breeding season, birds may

experience stronger competition for prey during periods of high resource demand; however, it is likely that selection will continue to favour generalists that can track local prey availability and opportunistically exploit different prey groups.

We thank the bird ringers who led the SYLVIA ringing activities and assisted with collecting warbler faecal samples. Special thanks go to Guillem Arrufat Tena and Oriol Clarabuch at Mas del Matà (Aiguamolls de l'Empordà), Marcel Gil-Velasco and Javier Oliver-Alejos (Llobregat Delta; Estany de Cal Tet and El Remolar-Filipines). Thanks also go to the reserve staff at each of the study sites for their assistance with securing permissions and access. The authors thank the ICO for all their support and help with the study including providing ringing contacts, permitting the use of ringing records in this study and arranging for the translation of the abstract into Catalan. Thanks go to the translators Raül Aymí (ICO) and Andreu Carretero. We also thank Jordan Cuff and Kirsty Franklin for their guidance during molecular lab work, Georgina Smethurst and Angela Marchbank for assistance with high-throughput sequencing and Lorna Drake for providing bioinformatics recommendations, template pipelines and data clean-up protocols. Finally, the authors thank the reviewers of this research, whose constructive feedback was instrumental in strengthening the manuscript.

AUTHOR CONTRIBUTIONS

S. R. Davies: Formal analysis; writing – original draft; methodology; investigation; conceptualization; validation; visualization; data curation; writing – review and editing; project administration. **I. P. Vaughan:** Conceptualization; investigation; funding acquisition; methodology; validation; writing – review and editing; supervision; formal analysis. **R. J. Thomas:** Supervision; formal analysis; validation; writing – review and editing; funding acquisition; investigation; conceptualization. **P. Josa:** Investigation; writing – review and editing; resources; methodology. **S. Bateman Posse:** Resources; methodology; investigation. **W. O. C. Symondson:** Conceptualization; investigation; funding acquisition; supervision; validation; project administration.

CONFLICT OF INTEREST STATEMENT

All authors declare that they have no conflicts of interest.

ETHICAL NOTE

All bird handling and ringing procedures were carried out by qualified and experienced bird ringers under licenses issued by the Catalan Institute of Ornithology (Institut Català d'Ornitologia, ICO) and the British Trust for Ornithology (BTO). Animal welfare guidelines were adhered to at all times, and every effort was made to minimise stress and handling time for all individual birds captured during mist-netting activities.

FUNDING

SRD was funded by the Natural Environment Research Council (NERC) GW4+ Doctoral Training Partnership (grant E/L002434/1).

DATA AVAILABILITY STATEMENT

The dataset and scripts that support the findings of this study are openly available on the Zenodo repository at <https://doi.org/10.5281/zenodo.18506241>.

REFERENCES

- Albrecht, M. & Gotelli, N.J. 2001. Spatial and temporal niche partitioning in grassland ants. *Oecologia* **126**: 134–141.
- Araújo, P.M., Lopes, P.B., da Silva, L.P. & Ramos, J.A. 2016. The importance of reedbeds and riparian areas for Cetti's warbler *Cettia cetti* throughout its annual cycle. *Wetlands* **36**: 875–887.
- Balança, G. & Schaub, M. 2005. Post-breeding migration ecology of reed *Acrocephalus scirpaceus*, moustached *A. melanopogon* and Cetti's warblers *Cettia cetti* at a Mediterranean stopover site. *Ardea* **93**: 245–257.
- Berzins, L.L., Mazer, A.K., Morrissey, C.A. & Clark, R.G. 2021. Pre-fledging quality and recruitment in an aerial insectivore reflect dynamics of insects, wetlands and climate. *Oecologia* **196**: 89–100.
- Bibby, C.J. & Green, R.E. 1983. Food and fattening of migrating warblers in some French marshlands. *Ring. Migr.* **4**: 175–184.
- Bibby, C.J. & Thomas, D.K. 1984. Sexual dimorphism in size, moult and movements of Cetti's warbler *Cettia cetti*. *Bird Study* **31**(1): 28–34.
- Bohmann, K., Evans, A., Gilbert, M.T.P., Carvalho, G.R., Creer, S., Knapp, M., Yu, D.W. & de Bruyn, M. 2014. Environmental DNA for wildlife biology and biodiversity monitoring. *Trends Ecol. Evol.* **29**: 358–367.
- Brambilla, M., Rizzoli, F., Franzoi, A., Caldonazzi, M., Zanghellini, S. & Pedrini, P. 2020. A network of small protected areas favoured generalist but not specialized wetland birds in a 30-year period. *Biol. Conserv.* **248**: 108699.

- Bussmann, C. 1979. Ökologische Sonderung der Rohrsänger Südfrankreichs aufgrund von Nahrungsstudien. *Vogelwarte* 30: 84–101.
- Camacho, C., Coulouris, G., Avagyan, V., Ma, N., Papadopoulos, J., Bealer, K. & Madden, T.L. 2009. BLAST+: architecture and applications. *BMC Bioinform.* 10: 1–9.
- Cardenas, A.M., Torres, J.A. & Bach, C. 1983. Estudio comparado del régimen alimentario de *Acrocephalus arundinaceus* y *A. scirpaceus* en la laguna de Zoñar. *Ardeola* 30: 33–34.
- Ceresa, F., Belda, E.J., Gómez, J., Miñana, I.J., Soler, L., Villarroja, J. & Monrós, J.S. 2016. Differences in time and space use between two sympatric *Acrocephalus* warblers with similar diets. *Bird Study* 63: 172–180.
- Clare, E.L., Symondson, W.O.C. & Fenton, M.B. 2014. An inordinate fondness for beetles? Variation in seasonal dietary preferences of night roosting big brown bats (*Eptesicus fuscus*). *Mol. Ecol.* 23: 3633–3647.
- Cramp, S. & Brooks, D. 1992. *The Birds of the Western Palearctic: Volume VI. Warblers*. Oxford: Oxford University Press.
- Crisol-Martínez, E., Moreno-Moyano, L.T., Wormington, K.R., Brown, P.H. & Stanley, D. 2016. Using next-generation sequencing to contrast the diet and explore pest-reduction services of sympatric bird species in macadamia orchards in Australia. *PLoS One* 11(3): e0150159.
- Cutting, K.A., Anderson, M.L., Beever, E.A., Schroff, S.R., Khaphake, E., Korb, N. & McWilliams, S. 2016. Niche shifts and energetic condition of songbirds in response to phenology of food-resource availability in a high-elevation sagebrush ecosystem. *Auk* 133: 685–697.
- Davies, S.R. 2020. *Projecting the effects of climate change on prey selection and dietary competition among Communities of European Reedbed Warblers*. PhD thesis, Cardiff University.
- Davies, S.R., Vaughan, I.P., Thomas, R.J., Drake, L.E., Marchbank, A. & Symondson, W.O.C. 2022. Seasonal and ontological variation in diet and age-related differences in prey choice, by an insectivorous songbird. *Ecol. Evol.* 12: e9180.
- Di Cecco, G.J. & Hurlbert, A.H. 2022. Multiple dimensions of niche specialization explain changes in species' range area, occupancy and population size. *Front. Ecol. Evol.* 10: 921480.
- Diamond, J.M. 1973. Distributional ecology of New Guinea birds: recent ecological and biogeographical theories can be tested on the bird communities of New Guinea. *Science* 179: 759–769.
- Drake, L.E., Cuff, J.P., Young, R.E., Marchbank, A., Chadwick, E.A. & Symondson, W.O.C. 2021. An assessment of minimum sequence copy thresholds for identifying and reducing the prevalence of artefacts in dietary metabarcoding data. *Methods Ecol. Evol.* 13: 694–710.
- Dyrce, A. 1981. Breeding ecology of great reed warbler *Acrocephalus arundinaceus* and reed warbler *Acrocephalus scirpaceus* at fish-ponds in SW Poland and lakes in NW Switzerland. *Acta Ornithol.* 18: 307–334.
- Dyrce, A. & Flinks, H. 2000. Potential food resources and nestling food in the great reed warbler (*Acrocephalus arundinaceus*) and the eastern great reed warbler (*Acrocephalus arundinaceus orientalis*). *J. Ornithol.* 141: 351–360.
- Fehlinger, L., Misteli, B., Morant, D., Juvigny-Khenafou, N., Cunillera-Montcusi, D., Chaguaceda, F., Stamenković, O., Fahy, J., Kolář, V., Halabowski, D., Nash, L.M., Jakobsson, E., Nava, V., Tirozzi, P., Urrutia Cordero, P., Mocq, J., Camacho Santamans, A., Zamora, J.M., Marle, P., Chonova, T., Bonacina, L., Mathieu-Resuge, M., Suarez, E., Osakpolor, S.E., Timoner, P., Evtimova, V., Nita, D., Carreira, B.M., Tapolczai, K., Martelo, J., Gerber, R., Dinu, V., Henriques, J., Selmezy, G.B. & Rimcheska, B. 2022. The ecological role of permanent ponds in Europe: a review of dietary linkages to terrestrial ecosystems via emerging insects. *Inland Waters* 13: 30–46.
- Ferreiro-Carballal, S., Belda, E.J., Ceresa, F. & Monrós, J.S. 2017. Dietary characterization of Savi's warbler *Locustella luscinioides* and great reed warbler *Acrocephalus arundinaceus* in eastern Spain. *Ardea* 105: 79–83.
- Fontanilles, P., Fourcade, J.M., de la Hera, I. & Kerbiriou, C. 2024. Passerines use of maize crop in addition to reedbed in autumn: abundance, diet and food availability in anthropogenic wetland. *Wetl. Ecol. Manag.* 32: 571–589.
- Freeman, B.G., Freeman, A.M.C. & Hochachka, W.M. 2016. Asymmetric interspecific aggression in new Guinean songbirds that replace one another along an elevational gradient. *Ibis* 158: 736–737.
- Gelman, A. & Hill, J. 2006. *Data Analysis Using Regression and Multilevel/Hierarchical Models*. Cambridge: Cambridge University Press.
- Gordon, R., Ivens, S., Ammerman, L.K., Fenton, M.B., Littlefair, J.E., Ratcliffe, J.M. & Clare, E.L. 2019. Molecular diet analysis finds an insectivorous desert bat community dominated by resource sharing despite diverse echolocation and foraging strategies. *Ecol. Evol.* 9: 3117–3129.
- Gotelli, N.J. & Ellison, A.M. 2013. *EcoSimR. Version 1.00*. Available at: <http://www.uvm.edu/~ngotelli/EcoSim/EcoSim.html>
- Halsch, C.A., Shapiro, A.M., Fordyce, J.A., Nice, C.C., Thorne, J.H., Waetjen, D.P. & Forister, M.L. 2021. Insects and recent climate change. *Proc. Natl. Acad. Sci. U. S. A.* 118: e2002543117.
- Harvey, W.G. 1977. Cetti's warblers in east Kent in 1975. *Br. Birds* 70: 96–107.
- Hiron, M., Rangel-Salazar, J.L. & Christensen, B. 2006. Coexistence of resident and migratory *Catharus* thrushes in a montane forest reserve of the northern Neotropics: on habitat use and morphology. *Ornithol. Neotrop.* 17: 383–394.
- Hixon, S.M., Sharma, B., Kainz, M.J., Wacker, A. & Arts, M.T. 2015. Production, distribution, and abundance of long-chain omega-3 polyunsaturated fatty acids: a fundamental dichotomy between freshwater and terrestrial ecosystems. *Environ. Rev.* 23: 414–424.
- Hoening, B.D., Snider, A.M., Forsman, A.M., Hobson, K.A., Latta, S.C., Miller, E.T., Polito, M.J., Powell, L.L., Rogers, S.L., Sherry, T.W., Toews, D.P.L., Welch, A.J., Taylor, S.S. & Porter, B.A. 2022. Current methods and future directions in avian diet analysis. *Auk/Ornithol.* 139: ukab077.
- Hoi, H. & Winkler, H. 1994. Predation on nests: a case of apparent competition. *Oecologia* 98: 436–440.
- Hoi, H., Eichler, T. & Dittami, J. 1991. Territorial spacing and interspecific competition in three species of reed warblers. *Oecologia* 87: 443–448.

- Holt, R.D. 1977. Predation, apparent competition, and the structure of prey communities. *Theor. Popul. Biol.* **12**: 197–229.
- Honza, M., Moksnes, A., Roskaft, E. & Oien, I.J. 1999. Effect of great reed warbler *Acrocephalus arundinaceus* on the reproductive tactics of the reed Warbler *A. scirpaceus*. *Ibis* **141**: 489–493.
- Hsieh, T.C., Ma, K.H. & Chao, A. 2016. iNEXT: an R package for rarefaction and extrapolation of species diversity (hill numbers). *Methods Ecol. Evol.* **7**: 1451–1456.
- van der Hut, R.M. 1985. Habitat choice and temporal differentiation in reed passerines of a Dutch marsh. *Ardea* **74**: 159–176.
- Ientile, R., Tuliozi, B., Campobello, D., Borghi, S., Sala, L., Dal Zotto, M. & Massa, B. 2022. Analysis of prey composition in Eurasian reed warblers' *Acrocephalus scirpaceus* droppings at four breeding sites in Italy. *Diversity* **14**: 1134.
- Iwaszkiewicz-Eggebrecht, E., Granqvist, E., Buczek, M., Prus, M., Kudlicka, J., Roslin, T., Tack, A.J.M., Andersson, A.F., Miraldo, A., Ronquist, F. & Łukasik, P. 2023. Optimizing insect metabarcoding using replicated mock communities. *Methods Ecol. Evol.* **14**: 1130–1146.
- Jiménez, J., Hernández, J.M., Feliú, J., Carrasco, M. & Moreno-Opo, R. 2018. Breeding in a dry wetland. Demographic response to drought in the common reed warbler *Acrocephalus scirpaceus*. *Ardeola* **65**: 247–259.
- Kent, C.M. & Sherry, T.W. 2020. Behavioral niche partitioning reexamined: do behavioral differences predict dietary differences in warblers? *Ecology* **101**: e03077.
- King, R.A., Symondson, W.O.C. & Thomas, R.J. 2015. Molecular analyses of faecal samples from birds to identify potential crop pests and useful biocontrol agents in natural areas. *Bull. Entomol. Res.* **105**: 261–272.
- Koma, Z., Grootes, M.W., Meijer, C.W., Nattino, F., Seijmonsbergen, A.C., Sierdsema, H., Foppen, R. & Kissling, W.D. 2021. Niche separation of wetland birds revealed from airborne laser scanning. *Ecography* **44**: 907–918.
- Kowarik, C., Martin-Creuzburg, D. & Robinson, C.T. 2021. Cross-ecosystem linkages: transfer of polyunsaturated fatty acids from streams to riparian spiders via emergent insects. *Front. Ecol. Evol.* **9**: 707570.
- Krüger, F., Clare, E.L., Greif, S., Siemers, B.M., Symondson, W.O.C. & Sommer, R.S. 2014. An integrative approach to detect subtle trophic niche differentiation in the sympatric trawling bat species *Myotis dasycneme* and *Myotis daubentonii*. *Mol. Ecol.* **23**: 3657–3671.
- Lack, D. 1976. *Island Biology Illustrated by the Land Birds of Jamaica*. Berkeley: University of California Press.
- Lam, M.M.-Y., Martin-Creuzburg, D., Rothhaupt, K.-O., Safi, K., Yohannes, E. & Salvarina, I. 2013. Tracking diet preferences of bats using stable isotope and fatty acid signatures of faeces. *PLoS One* **8**: e83452.
- Leisler, B. 1981. Die ökologische einnischung der mitteleuropäischen rohrsänger (*Acrocephalus*, Sylviinae). I. Habitattrennung. *Vogelwarte* **31**: 35–74.
- Leisler, B. 1991. *Acrocephalus arundinaceus*. In von Blotzheim, U.N. & Bauer, K. (eds) *Handbuch der Vögel Mitteleuropas*, Vol. **12/I**: 486–539. Wiesbaden: Aula.
- Leisler, B. & Schulze-Hagen, K. 2011. *Reed Warblers: Diversity in a Uniform Bird Family*: 321. Zeist: KNNV Publishing.
- Lenth, R. & Piaskowski, J. 2025. *emmeans: estimated marginal means, aka least-squares means*. R package version 2.0.1. Available at: <https://rvlenth.github.io/emmeans/>.
- Lewis-Phillips, J., Brooks, S.J., Sayer, C.D., Patmore, I.R., Hilton, G.M., Harrison, A., Robson, H. & Axmacher, J.C. 2020. Ponds as insect chimneys: restoring overgrown farmland ponds benefits birds through elevated productivity of emerging aquatic insects. *Biol. Conserv.* **241**: 108253.
- López-Iborra, G., Limiñana, R., Peñarrubia, S.G. & Pinheiro, T. 2005. Diet of common chiffchaffs *Phylloscopus collybita* wintering in a wetland in south-east Spain. *Rev. Catalana Ornitol.* **21**: 29–36.
- Lovette, I.J. & Hochachka, W.M. 2006. Simultaneous effects of phylogenetic niche conservatism and competition on avian community structure. *Ecology* **87**: S14–S28.
- MacArthur, R.H. 1958. Population ecology of some warblers of northeastern coniferous forests. *Ecology* **39**: 599–619.
- MacArthur, R.H. & Levins, R.A. 1967. The limiting similarity, convergence, and divergence of coexisting species. *Am. Nat.* **101**: 377–385.
- MacArthur, R.H. & Pianka, E.R. 1966. On optimal use of a patchy environment. *Am. Nat.* **100**: 603–609.
- Marchetti, C., Baldaccini, N.E. & Locatelli, D.M. 1996. Consistency and overlap of the diet of seven passerine trans-saharan migrants during spring stopover at two Mediterranean sites. *Ital. J. Zool.* **63**: 149–156.
- Martin-Creuzburg, D., Kowarik, C. & Straile, D. 2017. Cross-ecosystem fluxes: export of polyunsaturated fatty acids from aquatic to terrestrial ecosystems via emerging insects. *Sci. Total Environ.* **577**: 174–182.
- Martínez-Curci, N.S., Azpiroz, A.B., Isacch, J.P. & Elías, R. 2015. Dietary relationships among Nearctic and Neotropical migratory shorebirds in a key coastal wetland of South America. *Emu* **115**: 326–334.
- Martínez-Vilalta, J.M., Bertolero, A., Bigas, D., Paquet, J.Y. & Martínez-Vilalta, A. 2002. Habitat selection of passerine birds nesting in the Ebro Delta reedbeds (NE Spain): management implications. *Wetlands* **22**: 318–325.
- McMartin, B., Bellocq, M. & Smith, S. 2009. Patterns of consumption and diet differentiation for three breeding warbler species during a spruce budworm outbreak. *Auk* **119**: 216–220.
- Méró, T.O., Žuljević, A., Varga, K. & Lengyel, S. 2015. Habitat use and nesting success of the great reed warbler (*Acrocephalus arundinaceus*) in different reed habitats in Serbia. *Wilson J. Ornithol.* **127**: 477–485.
- Molina, J., Josa, A.H. & Camacho, I. 1998. Diet of Cetti's warblers *Cettia cetti* (Temminck, 1820) in a locality of southern Spain. *Ardeola* **45**: 217–220.
- Navalón, G., Bright, J.A., Marugán-Lobón, J. & Rayfield, E.J. 2019. The evolutionary relationship between beak shape, mechanical advantage, and feeding ecology in modern birds. *Evolution* **73**: 422–435.
- Nielsen, J.M., Clare, E.L., Hayden, B., Brett, M.T. & Kratina, P. 2018. Diet tracing in ecology: method comparison and selection. *Methods Ecol. Evol.* **9**: 278–291.
- Novakowski, G.C., Hahn, N.S. & Fugli, R. 2008. Diet seasonality and food overlap of the fish assemblage in a pantanal pond. *Neotrop. Ichthyol.* **6**: 567–576.
- Oksanen, J., Blanchet, F.G., Kindt, R., Legendre, P., Minchin, P.R., O'Hara, B., Solymos, P., Stevens, H. &

- Wagner, H.H. 2018. *vegan: community ecology package*. R package version 2.5-3. Available at: <https://CRAN.R-project.org/package=vegan/>.
- Orlowski, G. & Karg, J. 2013. Diet breadth and overlap in three sympatric aerial insectivorous birds at the same location. *Bird Study* **60**: 475–483.
- Perry, G. & Pianka, E.R. 1997. Animal foraging: past, present and future. *Trends Ecol. Evol.* **12**: 360–364.
- Petit, L.J., Petit, D.R., Petit, K.E. & Fleming, W.J. 1990. Annual variation in foraging ecology of Prothonotary warblers during the breeding season. *Auk* **107**: 146–152.
- Pianka, E.R. 1973. The structure of lizard communities. *Annu. Rev. Ecol. Syst.* **4**: 53–74.
- Pianka, E.R. 1974. Niche overlap and diffuse competition. *Proc. Natl. Acad. Sci. U. S. A.* **71**: 2141–2145.
- Pigot, A.L., Jetz, W., Sheard, C. & Tobias, J.A. 2018. The macroecological dynamics of species coexistence in birds. *Nat. Ecol. Evol.* **2**: 1112–1119.
- Pompanon, F., Deagle, B.E., Symondson, W.O.C., Brown, D.S., Jarman, S.N. & Taberlet, P. 2012. Who is eating what? Diet assessment using next generation sequencing. *Mol. Ecol.* **21**: 1931–1950.
- Poulin, B. & Lefebvre, G. 1996. Dietary relationships of migrant and resident birds from a humid forest in Central Panama. *Auk* **113**: 277–287.
- Poulin, B., Lefebvre, G. & Mauchamp, A. 2002. Habitat requirements of passerines and reedbed management in southern France. *Biol. Conserv.* **107**: 315–325.
- Pulliam, H.R. 1985. Foraging efficiency, resource partitioning, and the coexistence of sparrow species. *Ecology* **66**: 1829–1836.
- R Core Team 2021. *R: A Language and Environment for Statistical Computing*. Vienna, Austria: R Foundation for Statistical Computing. Available at: <https://www.R-project.org/>.
- Robinson, B.W. & Wilson, D.S. 1998. Optimal foraging, specialization, and a solution to Liem's paradox. *Am. Nat.* **151**: 223–235.
- Rosenberg, K.V., Ohmart, R.D. & Anderson, B.W. 1982. Community organization of riparian breeding birds: response to an annual resource peak. *Auk* **99**: 260–274.
- Rotenberry, J.T. 1980. Dietary relationships among shrubsteppe passerine birds: competition or opportunism in a variable environment. *Ecol. Monogr.* **50**: 93–110.
- Salinas-Ramos, V.B., Herrera Montalvo, L.G. & León-Regagnon, V. 2015. Dietary overlap and seasonality in three species of mormoopid bats from a tropical dry forest. *Mol. Ecol.* **24**: 5296–5307.
- Salsamendi, E., Garin, I., Arostegui, I., Goiti, U. & Aihartza, J. 2012. What mechanism of niche segregation allows the coexistence of sympatric sibling rhinolophid bats? *Front. Zool.* **9**: 1–12.
- Schaefer, T., Ledebur, G., Beier, J. & Leisler, B. 2006. Reproductive responses of two related coexisting songbird species to environmental changes: global warming, competition, and population sizes. *J. Ornithol.* **147**: 47–56.
- Schnell, I.B., Bohmann, K. & Gilbert, M.T. 2015. Tag jumps illuminated-reducing sequence-to-sample misidentifications in metabarcoding studies. *Mol. Ecol. Resour.* **15**: 1289–1303.
- Schoener, T.W. 1974a. Competition and the form of habitat shift. *Theor. Popul. Biol.* **6**: 265–307.
- Schoener, T.W. 1974b. Resource partitioning in ecological communities. *Science* **185**: 27–39.
- Schulze-Hagen, K. 1991. *Acrocephalus scirpaceus*. In Glutz von Blotzheim, U.N. & Bauer, K. (eds) *Handbuch der Vögel Mitteleuropas*, Vol. **12/I**: 433–486. Wiesbaden: Aula.
- Sherry, T.W., Johnson, M.D., Williams, K.A., Kaban, J.D., McAvoy, C.K. & Hallauer, A.M. 2016. Dietary opportunism, resource partitioning and consumption of coffee berry borers by five species of migratory wood warblers (Parulidae) wintering in Jamaican shade coffee plantations. *J. Field Ornithol.* **87**: 273–292.
- Shokralla, S., Spall, J.L., Gibson, J.F. & Hajibabaei, M. 2012. Next-generation sequencing technologies for environmental DNA research. *Mol. Ecol.* **21**: 1794–1805.
- Spence, A.R., Rankin, E.E.W. & Tingley, M.W. 2021. DNA metabarcoding reveals broadly overlapping diets in three sympatric north American hummingbirds. *Ornithology* **139**: 1–14.
- Stenhouse, E.H., Bellamy, P., Kirby, W., Vaughan, I.P., Drake, L.E., Marchbank, A., Workman, T., Symondson, W.O.C. & Orozco-terWengel, P. 2023. Multi-marker DNA metabarcoding reveals spatial and sexual variation in the diet of a scarce woodland bird. *Ecol. Evol.* **13**: e10089.
- Symondson, W.O.C. 2002. Molecular identification of prey in predator diets. *Mol. Ecol.* **11**: 627–641.
- Thomas, D.K. 1984. Feeding ecology and habitat selection in the reed warbler. *Ibis* **126**: 454–484.
- Trevelline, B.K., Latta, S.C., Marshall, L.C., Nuttle, T. & Porter, B.A. 2016. Molecular analysis of nestling diet in a long-distance Neotropical migrant, the Louisiana Waterthrush (*Parkesia motacilla*). *Auk* **133**: 415–428.
- Trevelline, B.K., Nuttle, T., Porter, B.A., Brouwer, N.L., Hoenig, B.D., Steffensmeier, Z.D. & Latta, S.C. 2018a. Stream acidification and reduced aquatic prey availability are associated with dietary shifts in an obligate riparian Neotropical migratory songbird. *PeerJ* **6**: e5141.
- Trevelline, B.K., Nuttle, T., Hoenig, B.D., Brouwer, N., Porter, B.A. & Latta, S.C. 2018b. DNA metabarcoding of nestling faeces reveals provisioning of aquatic prey and resource partitioning among Neotropical migratory songbirds in a riparian habitat. *Oecologia* **187**: 85–98.
- Turner, A.K. 1982. Optimal foraging by the swallow (*Hirundo rustica*, L): prey size selection. *Anim. Behav.* **30**: 862–872.
- Twining, C.W., Shipley, J.R. & Winkler, D.W. 2018. Aquatic insects rich in omega-3 fatty acids drive breeding success in a widespread bird. *Ecol. Lett.* **21**: 1812–1820.
- Twining, C.W., Brenna, J.T., Lawrence, P., Winkler, D.W., Flecker, A.S. & Hairston, N.G. Jr. 2019. Aquatic and terrestrial resources are not nutritionally reciprocal for consumers. *Funct. Ecol.* **33**: 2042–2052.
- Twining, C.W., Parmar, T.P., Mathieu-Resuge, M., Kainz, M.J., Shipley, J.R. & Martin-Creuzburg, D.M. 2021. Use of fatty acids from aquatic prey varies with foraging strategy. *Front. Ecol. Evol.* **9**: 735350.
- Valentini, A., Miquel, C., Nawaz, M.A., Bellemain, E., Coissac, E., Pompanon, F., Gielly, L., Cruaud, C., Nascetti, G., Wincker, P., Swenson, J.E. & Taberlet, P. 2009. New perspectives in diet analysis based on DNA barcoding and parallel pyrosequencing: the trnL approach. *Mol. Ecol. Resour.* **9**: 51–60.
- Vesterinen, E.J., Puisto, A.I.E., Blomberg, A.S. & Lilley, T.M. 2018. Table for five, please: Dietary partitioning in boreal bats. *Ecol. Evol.* **8**: 10914–10937.

- Vickery, J.A., Ewing, S.R., Smith, K.W., Pain, D.J., Bairlein, F., Skorpilová, J. & Gregory, R.D. 2014. The decline of afro-Palaearctic migrants and an assessment of potential causes. *Ibis* **156**: 1–22.
- Wagner, D.L., Grames, E.M., Forister, M.L., Berenbaum, M.R. & Stopak, D. 2021. Insect decline in the Anthropocene: death by a thousand cuts. *Proc. Natl. Acad. Sci. U. S. A.* **118**: e2023989118.
- Wang, Y., Naumann, U., Wright, S.T. & Warton, D.I. 2012. Mvabund – an R package for model-based analysis of multivariate abundance data. *Methods Ecol. Evol.* **3**: 471–474.
- Wiens, J.A. 1977. On competition and variable environments. *Am. Sci.* **65**: 590–597.
- Wiens, J.A. & Rotenberry, J.T. 1979. Diet niche relationships among north American grassland and shrubsteppe birds. *Oecologia* **42**: 253–292.
- Wilson, R.P. 2010. Resource partitioning and niche hypervolume overlap in free-living pygoscelid penguins. *Funct. Ecol.* **24**: 646–657.
- Zurdo, J., Barrero, A., Da Silva, L.P., Bustillo-de la Rosa, D., Gómez-Castasús, J., Morales, M.B., Traba, J. & Mata, V.A. 2023a. Dietary niche overlap and resource partitioning among six steppe passerines of Central Spain using DNA metabarcoding. *Ibis* **165**: 905–923.
- Zurdo, J., Reverter, M., Barrero, A., Bustillo-de la Rosa, D., Gómez-Castasús, J., Pérez-Granados, B., Morales, M.B. & Traba, J. 2023b. Prey choice in insectivorous steppe passerines: new insights from DNA metabarcoding. *Glob. Ecol. Conserv.* **48**: e02738.

Received 29 August 2025;

Revision 10 July 2026;

revision accepted 22 July 2026.

Associate Editor: Andrea Soriano-Redondo

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Data S1–S6 Details of resources and methods used in the molecular analysis and bioinformatics pipeline. Included as a separate Word document.

Data S7 Matrix of dietary samples $\times$ dietary taxa, and calculated frequencies of prey taxa occurrence for each warbler species consumer. Each unique prey taxon is reported within a new row, alongside its estimated body length in mm (from the literature) and habitat status (terrestrial or aquatic/semi-aquatic). Provided as a separate Excel spreadsheet.

Data S8 Breakdown of all warbler diet samples included in the statistical analyses. For each month and study site, the number of samples where the diet data were successfully retrieved is provided for each warbler species. Included as a separate Word document.

Data S9–S12 Presentation of results from additional analyses, including (i) test statistics from an alternative aquatic prey GLM where outliers were removed, (ii) bootstrap assessment of dietary overlap robustness to uneven sampling, (iii) plots of coverage-based rarefaction and extrapolation curves comparing Shannon-based dietary niche breadth ($q = 1$) with Simpson-based dietary niche breadth ($q = 2$) and (iv) *manyglm* test statistics from diet matrices collapsed at prey family and order levels. Included as a separate Word document.