POPULATION ECOLOGY – ORIGINAL RESEARCH

Seasonal variation of population and individual dietary niche in the avivorous bat, *Ia io*

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Abstract

The variation in niche breadth can affect how species respond to environmental and resource changes. However, there is still no clear understanding of how seasonal variability in food resources impacts the variation of individual dietary diversity, thereby affecting the dynamics of a population's dietary niche breadth. Optimal foraging theory (OFT) and the niche variation hypothesis (NVH) predict that when food resources are limited, the population niche breadth will widen or narrow due to increased within-individual dietary diversity and individual specialization or reduced within-individual dietary diversity, respectively. Here, we used DNA metabarcoding to examine the composition and seasonality of diets of the avivorous bat *Ia io.* Furthermore, we investigated how the dietary niches changed among seasons and how the population niche breadth changed when the availability of insect resources was reduced in autumn. We found that there was differentiation in dietary niches among seasons and a low degree of overlap, and the decrease of insect resource availability and the emergence of ecological opportunities of nocturnal migratory birds might drive dietary niche shifts toward birds in *I. io*. However, the population's dietary niche breadth did not broaden by increasing the within-individual dietary diversity or individual specialization, but rather became narrower by reducing dietary diversity via predation on bird resources that served as an ecological opportunity when insect resources were scarce in autumn. Our findings were consistent with the predictions of OFT, because birds as prey for bats provided extremely different resources from those of insects in size and nutritional value. Our work highlights the importance of size and quality of prey resources along with other factors (i.e., physiological, behavioral, and life-history traits) in dietary niche variation.

Keywords Bats · DNA metabarcoding · Individual specialization · Optimal foraging theory · Resource changes · Resource quality

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Introduction

Understanding the variability of niche breadth is fundamental for understanding patterns of biological adaptation, speciation, and range shifts (Sexton et al. 2017). The plasticity of niche breadth can affect how species respond to climatic, environmental, and resource changes. Food resource changes play a pivotal role in the variation of niche breadth. With the seasonality of natural environments, the availability of food resources fluctuates in response to environmental factors such as intra-annual climatic changes (Costa-Pereira et al. 2017; Willson et al. 2010). A typical seasonal ecosystem presents a rainy period with high abundance and diversity of food resources and a dry season with low food availability (Tonkin et al. 2017; Varpe 2017). Likewise, seasonal environmental changes cause the variety and availability of

food resources to be reduced in autumn and winter compared with spring and summer. For example, arthropod diversity and abundance were high in spring and summer and lower in autumn and winter in a highly seasonal desert (Vonshak et al. 2009). Such seasonal variation in food resources can be expected to affect individual diet variation and hence the population niche breadth of animals.

Classical optimal foraging theory (OFT) postulates that individuals will begin to include novel or alternative available resources in their diet when food resources become scarce. As a consequence, within-individual dietary diversity will increase, and the population niche breadth will expand (MacArthur and Pianka 1966; Perry and Pianka 1977). For example, one study found that when food became limited, moose individuals (*Alces alces*) added novel food items to their diet, thereby increasing their dietary niche breadth (Jesmer et al. 2020). However, OFT can make different predictions depending on the type of food and foraging behavior. OFT also states that when more energetically high-ranking and larger prey are available, the within-individual dietary diversity should decrease, and thus the niche breadth will become narrower. Therefore, one would expect that when food resources are depleted in autumn and winter, individuals will either forage on other prey items and thereby increase the population niche breadth, or else will forage on alternative high-quality and large-sized food resources, thus reducing the population niche breadth. Although the relationships between resource changes and dietary niche variation have been tested in many taxa, such as insects (Hardy et al. 2020), fish (Bolnick et al. 2007), amphibians (Costa-Pereira et al. 2019), reptiles (Costa et al. 2008), birds (Maldonado et al. 2017) and mammals (Karanth and Sunquist 1995), the idea is still debated. In fact, a meta-analysis of 12 terrestrial carnivores showed that dietary niche breadth was only positively related to increasing prey richness for six species (Ferretti et al. 2020). Thus, there is still no complete understanding of how seasonal variability in food resources affects the seasonal dynamics of individual diets, thereby affecting the population niche breadth. Therefore, it is necessary to investigate the relationships in more taxa, especially in species with a wider spectrum of available resources (e.g., bats).

Changes in the dietary niches of a population would ultimately depend on the dynamics of individual dietary niches such as individual niche variation or individual dietary specialization (Araújo et al. 2011; Bolnick et al. 2003; Bolnick et al. 2011; Forsman and Wennersten 2016). The niche variation hypothesis (NVH) also proposes that a population with wider dietary niches should exhibit higher among-individual dietary diversity (stronger individual specialization) in resource use (Van Valen 1965). When food resource availability was low, for instance, dietary niche breadth increased in a sea otter (*Enhydra lutris nereis*) population

due to increased individual dietary specialization (Tinker et al. 2008). Thus, understanding the ultimate drivers of individual specialization is crucial for revealing the process of population niche variation. Currently, a growing number of studies have investigated the relationships between individual diet variation and population niche breadth in fish (Costa-Pereira et al. 2017), birds (Jaeger et al. 2010) and mammals (Balme et al. 2020; Robertson et al. 2015). However, few studies have explored individual diet variation, dietary specialization, or their driving factors in nocturnal mammals due to their cryptic nature. Nocturnal animals occupy nocturnal niches (e.g., bats occupy a unique nocturnal niche and are the second largest group of mammals), and studying their diets and their dietary niche variation is important for understanding food webs, trophic interactions, and niche evolution in ecosystems (Burgar et al. 2014; Pompanon et al. 2012).

The avivorous bats represent excellent natural models that can be used to investigate dietary niche variation for several reasons. First, relative to other insectivorous species, avivory in bats represents a case of dietary shift or expansion from insects to birds. Only three bat species, *Nyctalus lasiopterus*, *Nyctalus aviator*, and *Ia io*, are seasonal aerial-hawking avivores (that is, bird-eating carnivores) and are known to hunt for insects in summer, but are also known to prey upon nocturnally migrating birds in both spring and autumn (Fukui et al. 2013; Gong et al. 2021a; Gong et al. 2022; Heim et al. 2019; Ibáñez et al. 2016, 2020). Second, the avivorous bats with a main distribution in the temperate and subtropical zones (Simmons 2005) thus experience seasonal fluctuation in resource availability such as decreases in the diversity and abundance of available insect resources in autumn, although the occurrence of nocturnally migrating birds presents an ecological opportunity (Han et al. 2007). Finally, the avivorous bats consuming a wider spectrum of prey resources from insects to birds that display large differences in body size, flight altitude and speed (El-Sayed 2019; Kerlinger and Moore 1989), and nutritional value (Popa-Lisseanu et al. 2007) may thereby provide an excellent natural model with which to investigate dietary niche variation.

The great evening bat *I. io* is currently the only known avivorous bat to catch birds on the wing in southern China. Previous studies using morphological identification methods have documented a seasonal change in the diet of *I. io* in response to local potential insect resources; the availability of insect resources was lower in autumn than in spring and summer (Han et al. 2007). The bats mainly prey on birds in spring and autumn, and feed on insects in summer, while in winter they either hibernate in caves or migrate south to overwinter elsewhere (Gong et al. 2021a; Han et al. 2007; Thabah et al. 2007). The present study employs DNA metabarcoding to determine the individual- and population-level dietary composition and seasonal changes in the avivorous

bat *I. io*. Furthermore, we explore dietary niche similarity and breadth among seasons and ask whether the population niche breadth broadened when the availability of insect resources was reduced in autumn compared with spring and summer. Third, if this is the case, we hypothesize that population niche breadth broadening in autumn will result from increases in individual dietary diversity (i.e., adding novel food items and/or ecological opportunity of bird resources) based on the prediction of OFT, or increases in individual dietary specialization based on the predictions of the NVH. Fourth, if this is not the case, we hypothesize that population niche breadth narrowing in autumn will be due to individuals switching to large and high-quality prey (i.e., birds in the case of the bat diet) based on another prediction of OFT. Finally, we aim to reveal whether phenotypic attributes (body mass), dietary diversity, and ecological opportunity affect individual dietary specialization in *I. io*.

Materials and methods

Study site, sample collection, and insect survey

The sampling was performed at Feilong Cave (24°58.43′N, 104°52.69′E) in Xingyi City, Guizhou Province, China. This area has a subtropical humid monsoon climate with four distinct seasons. The surrounding vegetation includes evergreen broad-leaved forest, coniferous forest, shrub, and grassland (Han et al. 2007; Han and He 2012). The diversity and abundance of insect resources present a seasonal pattern in response to local climate and environmental changes. In spring and summer, the availability of potential insect resources is high, while there is a clear decrease in autumn (Han et al. 2007).

Ia io is the most common bat species in the karst cave almost all year round. In winter, the bats either hibernate in the cave or migrate farther south to overwinter elsewhere (Gong et al. 2021a). Several bat species such as *Hipposideros pratti*, *Hipposideros armiger*, and *Myotis chinensis* also roost seasonally in this cave. Bat capture, fecal collection and preservation, and marking for individual identification followed the methods described by Gong et al. (2021a). Briefly, bats were captured with mist nets at cave entrances when they returned from nightly feeding. Each bat was placed separately in a clean and sterilized cotton bag (less than two hours) for fecal collection. The fecal pellets were placed in 2 ml cryo-tubes containing 100% ethanol and were individually labeled, and subsequently transported to the laboratory on dry ice and stored at − 20 °C. Bats were finally tagged with bat rings on the forearm for individual identification before being released into the cave. Any recaptured individuals were excluded from fecal collection within the same season. A total of 108 individual bat fecal

samples were collected from March to November 2017. The fecal samples were divided into three seasons based on seasonal climatic periods in Guizhou (Zhang et al. 2014): spring ($n = 31$, March to early May), summer ($n = 36$, June to August), and autumn ($n = 41$, September to November). Additionally, repeated samples in successive and separate seasons included four samples from spring resampled in summer, three from spring to autumn, seven from summer to autumn, and only two from three consecutive seasons.

To estimate the availability of insect food resources in the study area, we collected night-flying insects using light traps at five sites during the summer and autumn sampling periods. The distance between each pair of sampling sites was about 2 km. The trapping sites were the same in summer and autumn. The insect trap consisted of a 2.5 gallon polypropylene bucket, a 30 cm diameter plastic funnel with a smooth interior, and a 1000 W high-pressure mercury lamp suspended directly above the funnel. Insect traps were placed approximately 2 m above the ground at forest edges, grasslands, and agricultural fields. Insects were collected at each sampling site from 20:00 o'clock every night to 5:00 o'clock the next day. The captured insects were euthanized by placing 95% alcohol cotton balls in closed wide-mouth bottles, then transferred to paper bags and further sorted according to the two sampling periods of summer and autumn. All captured insect samples were assigned to the order level by an entomologist.

DNA extraction, PCR amplification, sequencing, and avian dataset acquisition

The prey DNA per bat was extracted from 150 to 180 mg fecal mixtures that were subsampled from homogenized fecal pellets of each bat using a QIAamp DNA Stool Mini Kit (Qiagen, Crawley, UK). The samples from each season were divided into two batches for extraction. An extraction blank was included at each extraction batch to check for cross-contamination. We used two primer pairs to amplify prey-specific mini-barcode regions simultaneously. For arthropod DNA analyses, a 225-bp long fragment of the cytochrome c oxidase subunit I (COI) barcode region was amplified using primers LCO-1490 (GGTCAACAAATCATAAAGATATTG G) and ZBJ-ArtR2c (WACTAATCAATTWCCAAATCC TCC) (Brown et al. 2014; Chang et al. 2019). For avian DNA analyses, a 380-bp fragment of the COI was amplified using a bird-specific primer pair COIPreyFW (CGA GCAGARCTAGGCCAACC) and COIPreyRW (GCAGGC GGTTTTATGTTGATTGCTG) (Pastor-Beviá et al. 2014). All PCR reactions were performed following the protocol in Brown et al. (2014). Each PCR batch included 1–2 PCR blanks. Three separate PCR replicates were conducted for each extract, and these were then pooled after PCR amplification. This strategy maximizes taxon detection, potentially

facilitates identification of sporadic contamination, and allows investigators to monitor any effects of variation in sample processing (Ficetola et al. 2015). PCR product visualization, purification, and quantification were performed according to Gong et al. (2021a). Of these 108 individual bats, 43 individuals with the feather-containing scats (9 in spring, 2 in summer, and 32 in autumn) could amplify the target band of bird-specific primers, and they were then sequenced and analyzed to estimate the bird prey diversity in the diet of *I. io* in our previous study (see the details in Gong et al. 2021a). For arthropod procedures, a specific combination of unique indices (a short sequence of bases) was attached to the primers to identify single samples after sequencing. Purified products were pooled in equimolar conditions, and paired-end sequencing (2×300) was done on an Illumina MiSeq platform (Illumina Inc., San Diego, CA, USA) following the standard protocols by Shanghai Majorbio Bio-Pharm Technology Co., Ltd., China.

Sequence analysis and taxonomic identification

Raw sequences were merged by FLASH (Magoč and Salzberg 2011) and quality-filtered by Trimmomatic (Bolger et al. 2014). The optimized sequences were obtained after quality control and then were clustered into molecular operational taxonomic units (MOTUs) at 97% similarity thresholds to generate an OTU table using Usearch with the singletons and chimera sequences removed (Edgar 2010). To remove low-abundance sequences and potentially erroneous sequences, those MOTUs with sequence numbers < 0.1% of the total sequences in each sample were discarded. The taxonomic identification of each MOTU was performed using the reference database in BOLD (http://www.boldsystems.org/) and a BLAST in Genbank (https://www.ncbi.nlm.nih.gov/genbank/) following the identification criteria of Aizpurua et al. (2018) and Alberdi et al. (2018). Order-level taxonomies were assigned at > 95% similarity values; family-level taxonomies were assigned at > 96.5%, and species-level taxonomies were assigned when the similarity values between the query and reference sequences were above 98%. All of the identified species were manually checked. When one MOTU matched multiple species that shared the highest matching score, we downgraded the taxonomic resolution to the most common level. Those MOTUs not fulfilling the taxonomy or not matching any reference sequence were classified as unidentified.

Statistical data analysis

All statistical analyses were conducted in R 3.5.0 (R Core Team 2018).

Dietary data analysis

Both the OTU tables of arthropods (present study) and birds (previous study by Gong et al. 2021a) were converted from the number of MOTU reads into presence/absence datasets and were merged into a single presence/absence OTU table for subsequent analysis. The prey composition in the diet of *I. io* among seasons was quantified using percent frequency of occurrence (%FOO: the percent of samples that contained a given prey taxon), percent of occurrence (POO: the number of given prey taxon occurrences divided by the total number of prey taxa occurrences), and weighted percent of occurrence (wPOO: weights each occurrence according to the number of prey taxa in the sample, e.g., if a sample contains three prey taxa, each will be given a weight of 1/3) of prey orders and families (Deagle et al. 2019). Here, we did not use the relative read abundance (RRA; Deagle et al. 2019) because merging the number of reads of both OTU tables from two pairs of primers would have a large deviation. Moreover, previous studies using the method have found that conclusions based on the presence/absence data are often qualitatively similar to those based on the RRA (Kartzinel et al. 2015; Pansu et al. 2018). We also constructed a predator–prey trophic network to visualize the seasonal dietary composition of *I. io* using the R package bipartite (Dormann et al. 2008).

We computed the species richness and Shannon diversity indices for identified prey of each bat individual among seasons using the R package vegan (Oksanen et al. 2019). Additionally, the consumed prey species diversity (at the MOTU level) of the *I. io* population among seasons was compared by calculating the interpolation and extrapolation curves based on Hill numbers $q = 0$ (species richness) and $q = 1$ (Shannon diversity) in the R package iNEXT (Hsieh et al. 2016) developed for unequal sampling effort and presence/absence data (incidence data). The 95% confidence intervals (CIs) were obtained by a bootstrap method based on 1,000 replications. The non-overlapping of 95% CIs indicates significant differences (Arrizabalaga-Escudero et al. 2018). Then, a Venn diagram was used to show the overlapping numbers of consumed prey species (MOTUs) by bat population in different seasons. We used a nonmetric multidimensional scaling (NMDS) with Bray–Curtis distance to examine the degree of diet similarity among seasons using the R package vegan (Oksanen et al. 2019). NMDS generates a stress value to quantify the effectiveness of an ordination for pattern analysis, with the value < 0.2 considered to be compliant (Clarke 1993). We assessed the degree of dietary overlap among seasons using Schoener's (1970) and Pianka's (1974) niche overlap indices. The two indices range from 0 (no overlap) to 1 (complete overlap), with values > 0.6 or < 0.4 generally considered as ecologically

significant correlations representing high or low levels of dietary overlap, respectively (Wallace 1981).

Measuring the population niche breadth and individual specialization

Dietary niche breadth at the population level for each season was computed by applying the Levins' index (Levins 1968) standardized (Levins' B_A) by Hurlbert (1978). Additionally, we also estimated total niche width (TNW) and within- and between-individual dietary diversity of the population for each season by using the Shannon–Weaver diversity index (Bolnick et al. 2003; Roughgarden 1972) in the R package RInSp (Zaccarelli et al. 2013). The TNW was partitioned into two components: the within-individual component, which is the average within-individual dietary diversity (WID), and the between-individual component, the average variation in among-individual dietary diversity (AID), such that TNW = WID + AID. Furthermore, the individual specialization (IS) in the diet was estimated by the ratio of WID to TNW (i.e., WID/TNW). The WID/TNW values vary between 0 and 1. As the values approach 0, this indicates decreases in between-individual overlap and thus a higher degree of IS; when the values approach 1, individuals consume a more complete range of food resources used by the population (Bolnick et al. 2002). Since only a WID/TNW ratio could be calculated for each season, the dietary IS for each individual in a single season was also quantified by calculating the proportional similarity index (PS_i, similarity between an individual dietary niche and the population dietary niche) (Feinsinger et al. 1981). The PS_i value varies from 1 (a dietary niche with complete similarity between the individual and population) to 0 (individuals use a subset of the population dietary niche). A lower PS_i value represents a stronger IS.

Statistical analyses

To quantify insect abundance during summer and autumn in the study area, we determined the mean number of collected insects from each order at five trapping sites. The Shannon diversity index was used to estimate insect diversity at different trapping sites. Independent sample t-tests were used to analyze the difference in insect diversity between summer and autumn. We compared differences in the %FOO, POO, and wPOO of prey consumed in the diet of *I. io* among seasons using a Chi-square test and a Kruskal–Wallis H test along with an associated post hoc Bonferroni analysis. The Kruskal–Wallis H test and post hoc Bonferroni test were also used to determine if species richness, Shannon diversity, and PS_i varied among seasons. The repeated samples in successive and separate seasons were excluded from the Kruskal–Wallis H test. Dietary differences among seasons

were tested using permutational multivariate analysis of variance (PERMANOVA) using the function adonis based on 999 permutations in the R package vegan (Oksanen et al. 2019). Additionally, we tested for pairwise differences between seasons using the pairwiseAdonis package (Arbizu 2020), and Bonferroni correction was used for multiple comparisons.

To test whether bird presence in the diet was associated with insect richness in the diets of individual bats, we coded presence/absence of birds in diet samples as 1/0 as a dependent variable, then performed a binary logistic regression analysis with insect richness as the independent variable.

To assess determinants of IS (measured by proportional similarity index, PS_i) in *I. io*, we employed a generalized linear mixed model (GLMM) with a beta distribution using the R package glmmTMB (Brooks et al. 2017). We excluded two data points that were related to rare occasions of bird hunting by *I. io* in summer, and thus data from 106 individual bats were included in the model analysis. We regarded the seasons in which bird resources appeared as ecological opportunity and defined the ecological opportunity as 1 in spring and autumn and 0 in summer. Additionally, we used the Shannon diversity index as a measure of dietary diversity because it reflected both the richness and evenness of prey species in the bat's diet. In the model, we used the PS_i as a dependent variable, the body mass of bats (BM), ecological opportunity of birds (EO), dietary Shannon diversity (DSD) as independent variables, and seasons and bat individuals as random factors. The GLMM validation was done with the R package DHARMa (Hartig 2022). Residuals were created using the simulateResiduals function with 1000 simulations. Residual dispersion was assessed with the testDispersion function, and the normal distribution of the residuals was confirmed using the testUniformity function. We also used Akaike's information criterion corrected for small sample size (AICc) to obtain the best-fitting GLMM using the R package MuMIn (Bartoń 2017). The GLMM with the smallest AICc value was identified as the best-fitting model. Finally, we conducted a hierarchical partitioning analysis that can alleviate multi-collinearity between predictor variables in the R package hier.part (Walsh and Mac Nally 2013) to estimate the independent contributions of the three predictor variables (Chevan and Sutherland 1991).

Results

Prey composition in the diet of *I. io* and insect abundance and diversity

After sequence processing, taxonomic identification, and avian dataset acquisition, we obtained a total of 436 MOTUs comprising the diet of 108 *I. io* individuals. Using arthropod

primers, we detected 12 prey orders (Blattodea, Coleoptera, Diptera, Hemiptera, Hymenoptera, Lepidoptera, Neuroptera, Odonata, Orthoptera, Trichoptera, Araneae, and Mesostigmata) from 410 MOTUs of arthropod prey items (Table S1). Additionally, our previous study showed that *I. io* consumed seven families of Passeriformes identified from 26 MOTUs of bird prey items (Gong et al. 2021a). Analysis of the population among seasons resulted in spring diets being characterized by 184 MOTUs, summer diets being characterized by 182 MOTUs, and autumn diets being characterized by 150 MOTUs.

We collected 6508 and 3009 insect samples from 12 and 7 different orders during summer and autumn, respectively, using insect traps in five trapping sites (Table S2). Diptera, Hemiptera, and Lepidoptera were the most frequent orders at each sampling area in summer, and Diptera was the most frequent order in autumn. The abundance of insect resources was higher in summer compared to autumn (Fig. 1a). The diversity of insect resources was significantly higher in summer than in autumn ($t = 7.665$, $P < 0.001$; Fig. 1b).
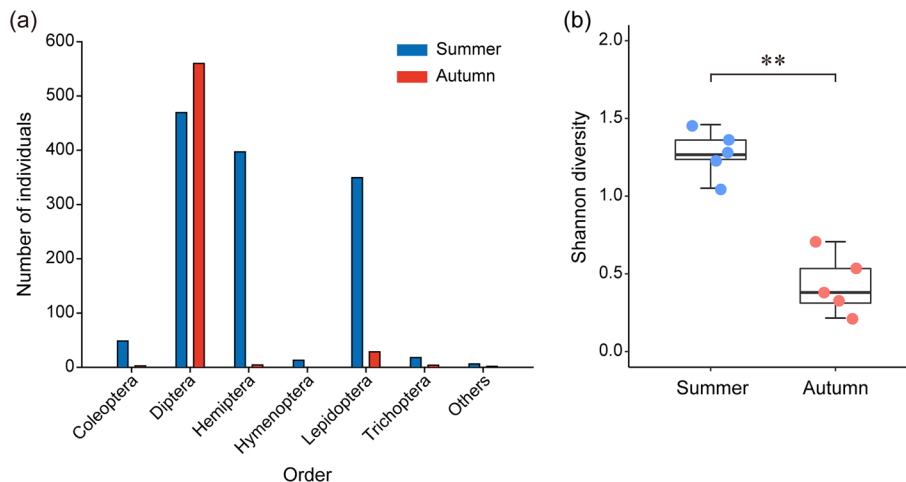
Seasonal dietary composition and variation

The diet of *I. io* showed strong seasonal variation (Figs. 2, 3). Significant differences among seasons in the frequency of consumption of Lepidoptera (%FOO: $\chi^2 = 33.810$, $P < 0.001$; POO: $\chi^2 = 8.556$, $P = 0.014$; wPOO: $H = 40.328$, $P < 0.001$), Coleoptera (%FOO: $\chi^2 = 14.519$, $P = 0.001$; POO: $\chi^2 = 13.770$, $P = 0.001$; wPOO: $H = 14.982$, $P = 0.001$), and Passeriformes (%FOO: $\chi^2 = 44.149$, $P < 0.001$; POO: $\chi^2 = 41.988$, $P < 0.001$; wPOO: $H = 52.400$, $P < 0.001$) were observed. In comparison with spring and summer, the consumption frequency of Lepidoptera (Chi-squared and Kruskal–Wallis H test with post hoc Bonferroni test, $P < 0.05$) and Coleoptera ($P < 0.05$) decreased significantly in autumn, while Passeriformes ($P < 0.05$) increased significantly in autumn (Figs. 2a, 3a, b). Similarly, at the

family level of Lepidoptera and Passeriformes, which were representative of the high frequency of overall consumption, the frequency of occurrence decreased significantly in the Sphingidae ($\chi^2 = 21.054$, $P < 0.001$) and Oecophoridae ($\chi^2 = 10.430$, $P = 0.005$), while that of Phylloscopidae ($\chi^2 = 35.786$, $P < 0.001$) and Muscicapidae ($\chi^2 = 12.025$, $P = 0.001$) increased significantly in autumn (Chi-squared with post hoc Bonferroni test, all $P < 0.05$) compared with spring and/or summer, although Cettiidae decreased significantly in autumn compared with spring ($\chi^2 = 3.989$, $P = 0.046$; Fig. 2b, c). Additionally, the trophic network structure between *I. io* and their prey showed that the composition of prey orders consumed differed among seasons. The diet of *I. io* was dominated by Lepidoptera and Diptera in spring, by Lepidoptera in summer, and by Passeriformes in autumn (Fig. 3c).

Seasonal dietary diversity, similarity, and overlap

Significant differences were observed in dietary diversity (species richness and Shannon diversity) at the individual and population levels among seasons (Fig. 4). At the individual level, dietary species (MOTU) richness was significantly higher in spring (12.29 ± 8.06; Kruskal–Wallis H test with post hoc Bonferroni test, $P = 0.022$) and summer (10.97 ± 5.28; $P = 0.037$) than in autumn (7.04 ± 6.75; Fig. 4a). Likewise, dietary Shannon diversity was significantly higher in spring (2.24 ± 0.84; $P = 0.022$) and summer (2.25 ± 0.60; $P = 0.037$) than in autumn (1.35 ± 1.19; Fig. 4b). At the population level, the 95% CIs for spring and summer did not overlap with autumn within the reference samples (i.e., within the sampling numbers), implying that the richness and diversity of consumed prey species in the *I. io* population was significantly higher in spring and summer than in autumn (Fig. 4c, d). We found that 24 prey MOTUs overlapped between spring and summer; 19 MOTUs overlapped between spring and autumn; 13 MOTUs overlapped

Fig. 1 Insect abundance (**a**) and Shannon diversity (**b**) in the study area during summer and autumn. Note that data from all five trapping sites were combined for this graph. Asterisks indicate a statistically significant difference (**$P < 0.01$)
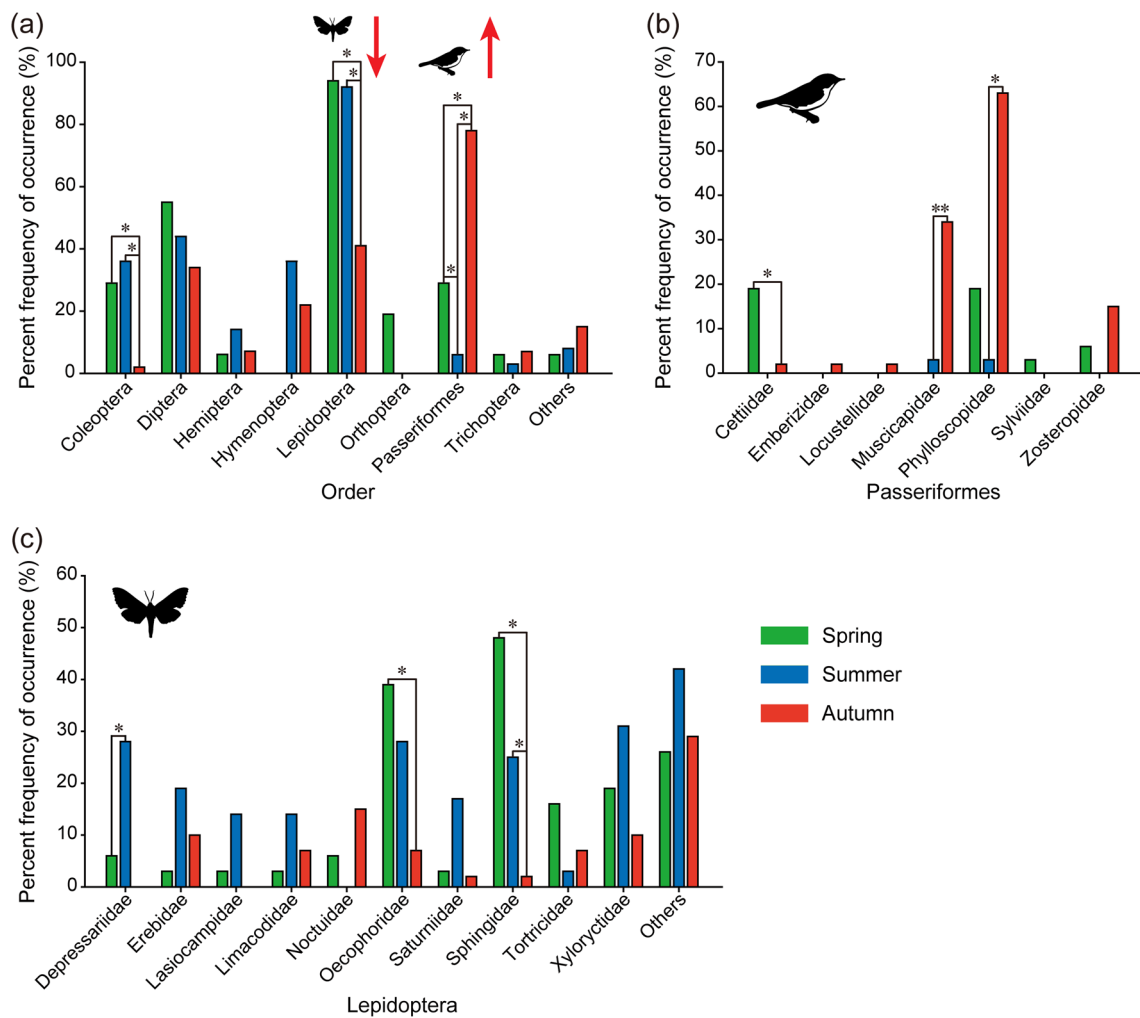
Fig. 2 Seasonal variation of dietary composition in *Ia io*. **a** Percent frequency of occurrence (%FOO) of prey orders in the diet. **b** %FOO of Passeriformes prey in the diet. **c** %FOO of Lepidopteran prey in the diet. Asterisks indicate a statistically significant difference (*$P < 0.05$, **$P < 0.01$)

between summer and autumn, while only 12 MOTUs were shared by the three seasons (Fig. 5a). The NMDS analysis showed a clear pattern in which samples were largely partitioned by seasons, suggesting that dietary composition varied among seasons (stress = 0.072 < 0.2; Fig. 5b). Meanwhile, the PERMANOVA results indicated that there were significant differences in the dietary composition of *I. io* among the three seasons (Pseudo-F = 6.079, $R^2 = 0.104$, $P = 0.001$), and significant differences were also observed in the pairwise comparisons between seasons (all $P < 0.05$; Table S3). These results suggested low dietary similarity among seasons. Additionally, Schoener's and Pianka's niche overlap indices were 0.135 and 0.167 between spring and summer, respectively; these were 0.108 and 0.138 between spring and autumn and 0.094 and 0.111 between summer and autumn (Table 1). These index values were all < 0.2, indicating low levels of dietary overlap in the bat population

in the three seasons. Importantly, we found that bird presence in the diet had a significant negative association with insect richness in bat diets ($\chi^2 = 11.574$, $P < 0.001$; Table 2 and Fig. S1).

Seasonal variation in population niche breadth and within- and among-individual dietary diversity

The widest dietary niche breadth of the *I. io* population was found in summer (Levins' $B_A = 0.631$, TNW = 4.988), followed by spring (Levins' $B_A = 0.539$, TNW = 4.905) and autumn (Levins' $B_A = 0.437$, TNW = 4.645; Table 1). The within-individual dietary diversity was greatest in spring (WID = 2.72), followed by summer (WID = 2.46) and autumn (WID = 2.42; Table 1). The greatest among-individual dietary variability was observed in summer (AID = 2.53) followed by autumn (AID = 2.23) and spring (AID = 2.19;

Fig. 3 Variation in dietary composition and predator–prey trophic network among seasons in *Ia io*. **a** Percent of occurrence (POO) of prey orders in the diet. **b** Weighted percent of occurrence (wPOO) of prey orders in the diet. **c** Predator–prey trophic network between *I. io* and their prey. In **c**, parallelograms connecting the upper and lower bars show the frequency of prey taxa (lower end of connector) in the diet of *I. io* (upper end of connector) among seasons
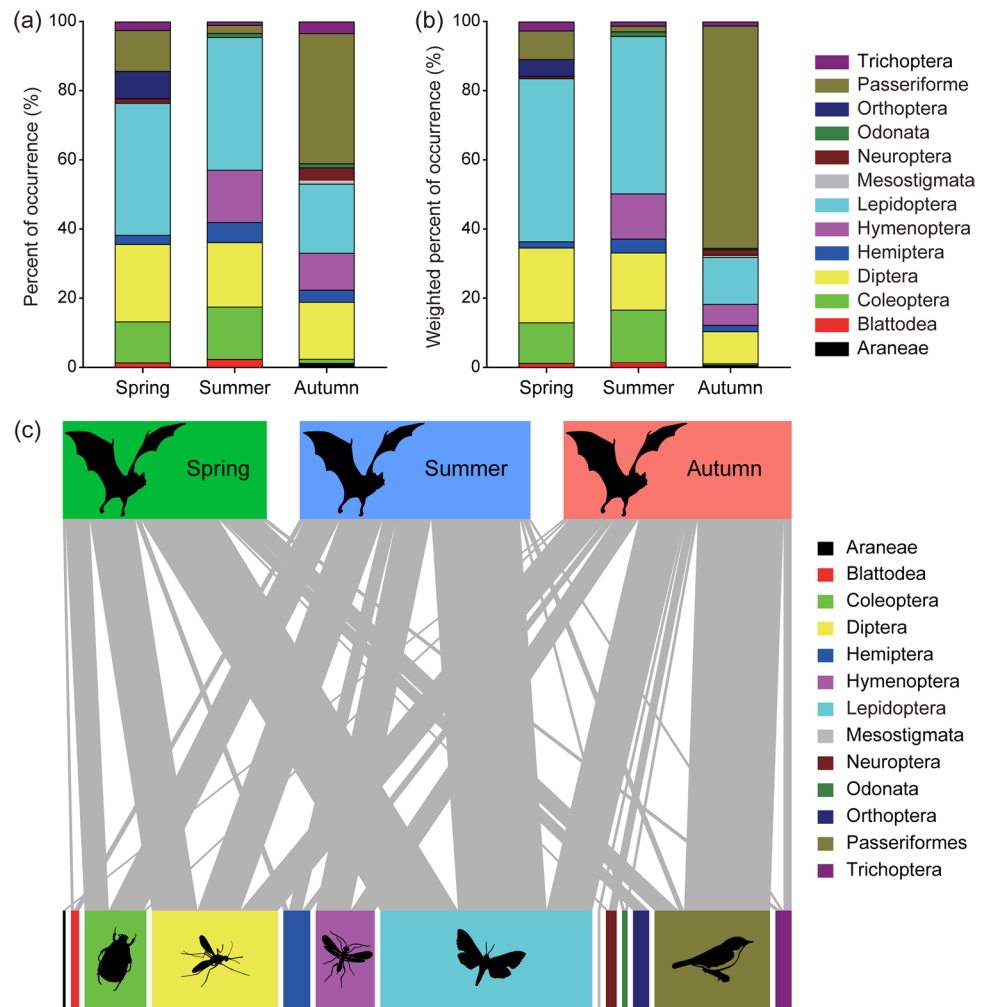
Table 1). The highest degree of IS (i.e., the lowest WID/TNW) was observed in summer (WID/TNW = 0.493), followed by autumn (WID/TNW = 0.521) and spring (WID/TNW = 0.554; Table 1). Additionally, there were no significant differences in PS_i values among seasons ($H = 2.040$, $P > 0.05$), but the mean PS_i value was slightly higher in spring, followed by autumn and summer (Table 1).

Factors influencing individual dietary specialization

The GLMM results showed that PS_i was significantly positively correlated with BM, EO, and DSD (Table 3). The residuals of the GLMM were normally distributed (Fig. S2). The best-fitting model (smallest AICc) also included the three predictors BM, EO, and DSD, and the full model did explain more variance than the null model (Table S4). Thus, individual bats with higher PS_i scores had heavier body mass and greater dietary diversity, and higher PS_i index values were measured in spring and autumn. Additionally, the results of the hierarchical partitioning analysis showed

that the independent contributions of BM, EO, and DSD were 1.854%, 9.797%, and 88.349%, respectively (Table 3).

Discussion

In this study, we found that the dietary composition of *I. io* showed clear seasonal changes. The proportion of avivorous individuals increased when insect availability (diversity and abundance) decreased and the presence of migratory birds increased in autumn, suggesting that both factors may together drive dietary niche shifts of *I. io* toward birds. Moreover, differentiation in the dietary niches among seasons was detected, and the dietary niche breadth was the widest in summer and the narrowest in autumn. When the availability of insect resources decreased in autumn, the population's dietary niche breadth did not broaden by increasing individual dietary diversity or individual dietary specialization, but rather became narrower by reducing dietary diversity due to individuals shifting to consuming large and high-quality bird prey, which supports our hypothesis based

Fig. 4 Seasonal variation of dietary diversity of *Ia io* at the individual and population levels. **a** Dietary MOTU (species) richness and **b** dietary Shannon diversity of *I. io* individuals among seasons. **c** Dietary species richness and **d** species diversity of the *I. io* population among seasons. In **a** and **b**, asterisks indicate a statistically significant difference (*$P < 0.05$); ns, not significant. In **c** and **d**, shaded areas represent 95% confidence intervals (CIs); the non-overlapping of 95% CIs indicates significant differences
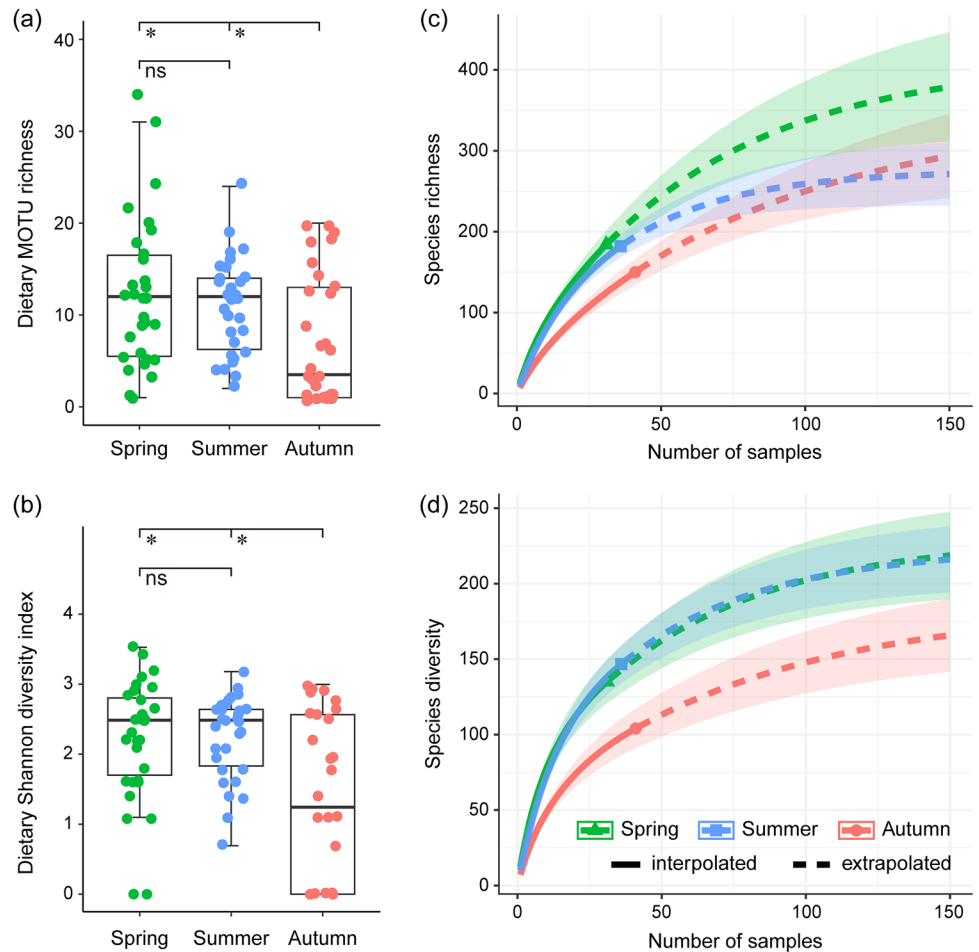
Fig. 5 Dietary overlap of the *Ia io* population among seasons. **a** The Venn diagram shows the overlapping numbers of consumed MOTUs. **b** The NMDS diagram shows the degree of overlap (similarity) of the diet in *I. io*. In **b**, ellipses represent 95% confidence intervals
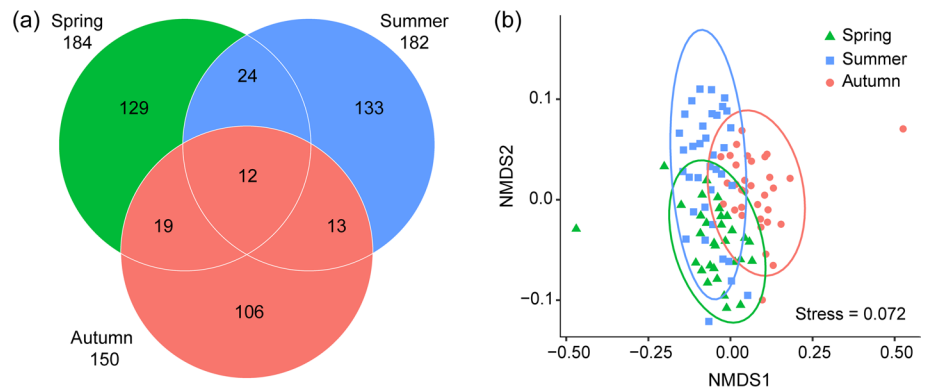
on the predictions of OFT. Finally, our results indicated that individual dietary specialization was driven by each bat's body mass, dietary diversity, and ecological opportunity.

Dietary composition and shifts

When compared with the previous studies (Han et al. 2007; Thabah et al. 2007), we found that the diet of *I. io* included a wider variety of insects (present study) and passerine birds (Gong et al. 2021a) and showed clear seasonal changes.

Previous studies based on morphological identification showed that the consumption frequency of arthropods by *I. io* was dominated by Coleoptera (Han et al. 2007; Thabah et al. 2007). However, the Lepidoptera dominated the frequency of occurrence of arthropods in the diet of *I. io* in our study. Morphological identification probably resulted in an overrepresentation of beetles because they have a hard carapace (Clare et al. 2014; Dodd et al. 2012); our data confirm this suggestion. Moreover, the molecular analyses of diets in bats may have some identification biases toward

Table 1 Dietary niche characteristics of the *Ia io* population among seasons

	Spring	Summer	Autumn
Schoener's (Pianka's) index	0.135 (0.167) 0.108 (0.138)	0.094 (0.111)	
Levins' B_A	0.539	0.631	0.437
TNW	4.905	4.988	4.645
WID	2.715	2.457	2.420
AID	2.190	2.531	2.225
WID/TNW	0.554	0.493	0.521
PS_i	0.124 ± 0.082	0.092 ± 0.052	0.115 ± 0.074

These characteristics include niche breadth (Levins' B_A), niche overlap (Schoener's and Pianka's indices), the population total niche width (TNW), within-individual dietary diversity (WID), among-individual dietary diversity (AID), and individual dietary specialization (WID/TNW and PS_i) of *Ia io* in three seasons. PS_i values are expressed as mean ± standard error

Lepidoptera (Clare et al. 2009; Salinas-Ramos et al. 2015), but it is not always the most abundant order. For example, the diet of *Eptesicus fuscus* showed a predominance of beetles (Coleoptera) (Clare et al. 2014), suggesting that this effect may slightly impact the dietary analysis. In our study, the proportion of avivorous individuals markedly increased in autumn, suggesting that a decrease in the availability of insects as food sources and the emergence of nocturnally migrating birds providing ecological opportunity may together promote the dietary shifts of *I. io* from insects to birds. Our finding was similar to the results found in two piscivorous bats, *Noctilio leporinus* and *Myotis pilosus*, indicating that dietary shifts occurred with a change from insects to fish during the seasons with lower insect prey resource availability (Brooke 1994; Ma et al. 2006).

The relationship between individual dietary diversity and population niche breadth

We found that the similarity of dietary niche of the *I. io* population was low in different seasons, suggesting that there was seasonal differentiation. Seasonal environmental changes cause seasonal variation in food resource availability, and thus drive dietary niche breadth variation. In our study area, the reduction of the availability of insect

Table 3 Generalized linear mixed models assessing the influence of body mass of bats (BM), ecological opportunity of birds (EO), dietary diversity (dietary Shannon diversity, DSD), and seasons and bat individuals as random factors on individual dietary specialization (measured by proportional similarity index, PS_i) of *Ia io*

	Estimate	SE	z	P	IF
(Intercept)	− 5.139	0.369	− 13.913	< 0.0001	–
BM	0.024	0.006	3.728	0.0002	1.854
EO	0.414	0.085	4.896	< 0.0001	9.797
DSD	0.722	0.050	14.510	< 0.0001	88.349

IF represents the independent contributions of factors to individual dietary specialization of *I. io* using hierarchical partitioning analysis

resources in autumn compared with spring and summer (Han et al. 2007) was due to local seasonal climate and environmental changes. Here, our survey of insect resources also showed a significant decrease in insect abundance and diversity in autumn compared to summer.

OFT predicts that population niche breadth will increase or narrow by changing individual dietary diversity as food resources become limited (MacArthur and Pianka 1966; Pyke et al. 1977). In this study, the dietary niche breadth of the population of *I. io* was the widest in summer, followed by spring, and the narrowest in autumn, showing that the population niche breadth did not broaden as insect resources became limiting in autumn. Moreover, we also found that the individual dietary diversity (i.e., dietary species richness, Shannon diversity, and WID) did not increase in autumn, and this might be one reason the population niche breadth did not broaden. Thus, our findings were inconsistent with the first hypothesis based on the prediction of OFT: broadening population niche breadth by adding novel food items and/or ecological opportunity in the form of bird resources. This may be due to the emergence of nocturnal migratory birds. Our results revealed that most individual bats (59%) only consumed birds in autumn, and bats preyed on birds whose body size was often an order of magnitude greater than those of insects; for example, an average of 7.6 g in a frequently preyed upon bird, *Phylloscopus inornatus* (Gong et al. 2021a), vs. an average of 1.5 g in moths of the Sphingidae. We also found that bird presence in the diet had a significant negative association with insect richness in *I. io* diets, supporting our suggestion. In addition, passerine

Table 2 Binary logistic regression analysis of insect richness related to the presence/absence of birds in the diet of *Ia io*

Factor	B	SE	Wald	df	P	Exp(B)	95% CI for Exp(B)
Insect richness	− 0.114	0.033	11.574	1	< 0.001	0.892	(0.836, 0.953)

B logistic coefficient, *SE* standard error of estimate, *Wald* Wald chi-square values, *df* degrees of freedom, *Exp(B)* exponentiated coefficient. *95.0% CI for EXP(B)* 95% confidence interval for Exp(B)

birds had higher nutritional value than invertebrate insects, in accordance with their superior position in the trophic chain (Popa-Lisseanu et al. 2007). The nutritional composition of insects and birds is rich in protein, fats, and amino acids. The average content of protein and fat was 66.9% and 11.1%, respectively, in 11 arthropod taxa (Razeng and Watson 2015), and 21.7% and 13.9% in some wild birds (Al-Amer et al. 2016; Khalifa and Nassar 2001; Woodward et al. 1995). This showed that the relative content of protein in arthropods was higher than that in some wild birds. However, the average amount of total amino acids (TAAs) and essential amino acids (EAAs) in arthropods was lower than that in some wild birds (74.6 g/100 g protein vs. 92.8 g/100 g protein in TAAs; 34.2 g/100 g protein vs. 37.8 g/100 g protein in EAAs) (Al-Amer et al. 2016; Khalifa and Nassar 2001; Landi et al. 2018; Rumpold and Schlüter 2013). Amino acid profiles are markers for protein quality (Huang et al. 2017; van Huis 2013). Thus, the relatively higher levels of TAAs and EAAs in birds may imply that they have higher quality protein than insects. Presumably, the high-quality protein and slightly higher fat content obtained by bats from preying on birds may be equivalent to many insects. Therefore, this may explain why individual bats do not increase dietary diversity to broaden population niche breadth in autumn, thus supporting our hypothesis based on another prediction of OFT: that population niche breadth will narrow due to individuals switching to large and high-quality bird prey.

The relationship between individual specialization and population niche breadth

The NVH proposes that populations with broader niches are achieved by an increase in the degree of IS as food resources become limited (Bolnick et al. 2003; Van Valen 1965). In accordance with the NVH, the population niche breadth increased both in sea otters (*E. lutris nereis*; Tinker et al. 2008) and the woolly mouse opossum (*Marmosa paraguayana*; Pires et al. 2013) when the diversity in food resource availability was low due to increases in the degree of IS. Here, the population niche breadth of *I. io* did not broaden by increasing IS when insect resources were scarce in autumn. This result did not support our hypothesis based on the predictions of the NVH. Insect diversity is extremely high compared with birds in nature, even in autumn. Our previous study found that *I. io* fed on 22 bird species (Gong et al. 2021a). In this case, most individuals (78%, including those that consumed only birds, and those that consumed both birds and insects) primarily preyed on birds in autumn, leading to relatively little among-individual variation in dietary diversity (i.e., more overlap in their diets) and thus a lower degree of IS,

leading to a narrower population niche breadth in autumn than in spring and summer.

Effects of other factors on dietary niche variation

We found that *I. io* preyed on many species of Passeriformes to attain their optimal nutritional diet in autumn before going into migration and hibernation. Consistent with a previous study (Machovsky-Capuska et al. 2018), our results suggest that the differences in nutritional values of the prey resources consumed by predators may affect seasonal variation in their dietary niche breadth. Additionally, individual variation in behavior, life-history traits, physiology, or morphology may enhance among-individual variation in diets to expand population niche breadth (Araujo et al. 2009; Bolnick et al. 2003; Sexton et al. 2017). Nutritional and energy requirements (i.e., physiological requirements) before entering hibernation and migration in autumn would encourage *I. io* to prey on more prey items. However, the larger body size and higher nutritional value of birds compared to insects means that it is difficult for *I. io* to broaden the dietary niche breadth, as bats prey on a bird that may be the size and nutritional equivalent of many insects. Our previous study demonstrated that *I. io* tend to prey on birds in autumn, resulting in a shift in the composition and function of gut microbiomes that can facilitate adaptation of energy and nutritional requirements for changes in foraging behavior and life-history traits (i.e., hibernation and migration) (Gong et al. 2021b). Thus, researchers should consider the size and nutritional value of food resources when explaining the variation of dietary niche breadth (Machovsky-Capuska et al. 2016; Raubenheimer et al. 2009). Taken together, these results suggest the importance of prey size and/or quality to individual dietary breadth.

The drivers of individual dietary specialization

Our results showed that IS was negatively related to dietary diversity, ecological opportunity, and the body mass of individual bats. The results were consistent with previous studies on fishes and mammals revealing that dietary diversity changes caused by resource variation and body condition affect IS (Balme et al. 2020; Bolnick and Ballare 2020; Robertson et al. 2015). First, in *I. io*, in accordance with the general theory that increases in individual dietary diversity result in higher similarity between the individual and the population in diet, IS was lower (i.e., higher PS_i). Moreover, ecological opportunity has given rise to high resource diversity leading to increased IS in leopards (*Panthera pardus*) (Balme et al. 2020). However, although ecological opportunity allowed *I. io* individuals to prey on a wider range of prey such as birds, no significant increase was observed in individual dietary diversity in autumn, since birds provided less

diversity while providing higher quality food than insects. In this case, the total resource diversity (insects + birds) of *I. io* would depend on insect resource diversity in autumn. Here, a decrease in insect resource diversity was observed in autumn, suggesting that the total resource diversity actually may not increase in autumn, and thus lead to a decrease in IS (higher PS_i) in *I. io*. Finally, in the European badger (*Meles meles*), the degree of IS had a positive relationship with body condition (Robertson et al. 2015). However, in our study, a larger body size of *I. io* was associated with a decrease in IS, possibly because more prey might be needed to compensate for the larger energy expenditure and demand of *I. io* with larger body size during fast flight. However, only further experimental examination will help in clarifying this relationship between prey diversity, energy expenditure, and body size in aerial insectivorous and avivorous bats. These results indicated that the incidence of IS may vary in different wild animal populations. To date, little research has focused on IS of bat species, and this topic deserves further attention.

Conclusions

In this study, we demonstrated that the dietary shift from insects to birds in *I. io* occurred because of low insect resource availability and the presence of nocturnal migratory birds. However, the population niche breadth did not broaden via increasing individual dietary diversity or the degree of IS, but rather narrowed as individuals switched to bird prey with a large body size and high nutritional value. This was consistent with the prediction of OFT that when food becomes limited, dietary niche breadth will decrease due to individuals switching to large and high-quality prey. These findings demonstrated that individuals may change foraging behavior in response to shifting ecological conditions and physiological requirements, thereby causing variation in individual diets and hence affecting the dietary niche dynamics of populations. Importantly, our work suggests the importance of size and quality of food resources as well as individual behavior, physiology, and life-history traits in dietary niche evolution. Future studies integrating multiple factors are needed to clarify the evolution of dietary niche breadth and the relative importance of each factor.

Data availability The datasets used in this study are available from the corresponding author on reasonable request. Representative sequences of each MOTU, and both OTU tables for arthropods and birds are available from the Dryad Digital Repository: https://doi.org/10.5061/dryad.bzkh1899m.

Declarations

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