Do the evolutionary interactions between moths and bats promote niche partitioning between bats and birds?

Lorinda Bullington[1], Mathew Seidensticker[2], Nathan Schwab[3], Philip Ramsey[4], and Kate Stone[4]

[1]University of Montana Missoula
[2]Northern Rockies Research & Educational Services
[3]Tetra Tech Inc Bothell Office
[4]MPG Ranch

April 16, 2024

Abstract

Ecological theory suggests that the coexistence of species is promoted by the partitioning of available resources, as in dietary niche partitioning where predators partitioning prey. Yet, the mechanisms underlying dietary niche partitioning are not always clear. We used fecal DNA barcoding to investigate diets of seven nocturnal insectivorous bird and bat species. Low diet overlap (2-22%) supported resource partitioning among all species. Differences in diet corresponded with species identity, prey detection method, and foraging behavior of predators. Insects with ultrasonic hearing capabilities were consumed significantly more often by birds than by bats, consistent with an evolved avoidance of echolocating strategies. In turn, bats consumed a greater proportion of non-eared insects such as spruce budworm. Overall, our results suggest that evolutionary interactions among bats and moths translate to dietary niche partitioning and coexistence among bats and nocturnal birds.

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Short running title: Diet partitioning of bats and birds

Lorinda Bullington[1,2*], Mathew T. Seidensticker[1,3], Nathan Schwab[4], Philip Ramsey[1], Kate Stone[1]

[1]MPG Ranch Missoula, MT, 59801, USA

[2]Department of Ecosystem and Conservation Sciences, University of Montana, Missoula, MT, 59812, USA

[3]Northern Rockies Research & Educational Services, P.O. Box 1242, Lolo, MT, 59847, USA

[4]Tetra Tech, 19803 North Creek Parkway, Bothell, WA 98011

*Corresponding author:

Lorinda Bullington

1001 S Higgins Ste. A3

Missoula, MT 59801

(406) 547-4413

L. Bullington: lbullington@MPGRanch.com

M. Seidensticker: mat@nrres.org

N. Schwab: *Nathan.Schwab@tetratech.com*

P. Ramsey: pramsey@mpgranch.com

K. Stone: kstone@mpgranch.com

Authorship

KS, MS, and NS designed the study and performed field collections. LB performed data analyses and wrote manuscript with edits and contributions from all coauthors.

Data accessibility

All barcode sequences are publicly available on BOLD under the datasets MPG and MPGR. Upon acceptance, OTU tables showing diet composition of all insectivores will be made available on Figshare.

ABSTRACT

Ecological theory suggests that the coexistence of species is promoted by the partitioning of available resources, as in dietary niche partitioning where predators partitioning prey. Yet, the mechanisms underlying dietary niche partitioning are not always clear. We used fecal DNA barcoding to investigate diets of seven nocturnal insectivorous bird and bat species. Low diet overlap (2-22%) supported resource partitioning among all species. Differences in diet corresponded with species identity, prey detection method, and foraging behavior of predators. Insects with ultrasonic hearing capabilities were consumed significantly more often by birds than by bats, consistent with an evolved avoidance of echolocating strategies. In turn, bats consumed a greater proportion of non-eared insects such as spruce budworm. Overall, our results suggest that evolutionary interactions among bats and moths translate to dietary niche partitioning and coexistence among bats and nocturnal birds.

INTRODUCTION

Aerial insectivores like birds and bats are decreasing at alarming rates across North America (Spiller & Dettmers, 2019), in part due to simultaneous declines of aerial insects (Sánchez-Bayo & Wyckhuys, 2019). Niche theory predicts that in resource-limited environments, species that occupy the same guild will partition dietary resources to avoid competitive exclusion (MacArthur & Levins, 1964). Such partitioning is often underpinned by variations in morphology or behavior that allow species to exploit different resources (Schoener, 1974). Dietary niche partitioning related to prey size (Vesterinen et al., 2018), predator morphology, and echolocation behavior (Emrich et al., 2014) is evident among many sympatric bat species. If and how dietary partitioning occurs among co-occurring nocturnal insectivorous birds and bats is less clear, but by identifying the processes that promote the coexistence of aerial insectivores, we can better predict future community dynamics.

Interactions between bats and moths provide a model system for studying the evolution of predator-prey relationships (Waters 2003; Hofstede and Ratcliffe 2016). Prey capture by bats is often dependent on echolocation behavior and how insects respond (Fenton and Fullard 1979). Moths with ultrasound-sensitive ears can hear echolocation calls at distances up to 100 meters (e.g., noctuids; Miller and Surlykke 2001) and avoid predation through evasive maneuvers or sounds (Dunning and Kruger, 1996). This adaptation arose independently in moths at least six times (Hofstede & Ratcliffe, 2016). In turn, some bats echolocate at low intensities or high enough frequencies to go undetected by moths (Faure, Fullard, and Barclay 1990; Hofstede and Ratcliffe 2016). Yet, how evolutionary interactions between moths and bats may extend to dietary resource partitioning between bats and nocturnal insectivorous birds is unknown (Yack et al., 2020). Nocturnal birds often use visual cues and possess adaptions for silent flight that enable them to evade detection by insects (Clark et al., 2020). These adaptations may allow them to exploit resources that bats cannot. For example, eared moths can only detect the cyclic wingbeats of approaching birds within 2.5 meters (Hofstede and Ratcliffe 2016; Waters 2003), perhaps making moths more vulnerable to predation by visually-oriented insectivores. The distributions of bats and nocturnal insectivorous birds suggests that they interact. However, little research exists on if, or to what extent they may partition prey resources, or the underlying mechanisms (Fenton and Fleming 1976).

We used fecal DNA barcoding to analyze the diets of seven co-occurring nocturnal aerial insectivores (hereafter NAIs). We compared the diet composition and richness of three nocturnal birds: *Chordeiles minor* (Common Nighthawks), *Phalaenoptilus nuttallii* (Common Poorwills), *Psiloscops flammeolus* (Flammulated Owls), and four bat species: *Eptesicus fuscus* (Big Brown Bats), *Lasionycteris noctivagans* (Silver-haired Bats), *Myotis Volans* (Long-legged Myotis), and *Myotis evotis* (Western Long-eared Myotis). Despite differences in prey detection methods used by these insectivores (Table 1), previous studies using microscopy of fecal samples have reported broad similarities in the insects they consume, primarily moths and beetles (Agosta, 2002; Csada et al., 1992; Ober & Hayes, 2008; Reynolds & Linkhart, 1987; Todd et al., 1998; Whitaker, 1995). However, NAI diets vary across regions and over time, hampering cross-study comparisons. Additionally, traditional methods of prey analysis in feces primarily result in prey identification to only the order or family level, which masks resource partitioning at finer taxonomic resolutions.

As with differences in prey detection methods, NAIs in this study also display different foraging behaviors. For example, Flammulated Owls (Goggans 1985) and Common Poorwills (Brigham and Barclay 1992) are sit-and-wait predators (Table 1). Both use their legs to launch after prey from the ground or perches, a foraging behavior not found in insectivorous bats. Modifications of the pelvis that allow bats to hang from perches and fly prevent bats from jumping into flight (Schutt et al., 1997). Instead, the bats in this study hunt by foraging insects while in flight, termed "aerial hawking" (Saunders & Barclay, 1992), or, as in Long-eared Myotis, sometimes by gleaning insects from the ground and foliage (Faure and Barclay 1994). Like bats, Common Nighthawks are also aerial hawkers and prey on insects at a wide range of heights above ground and over great distances in a single foraging bout (Clark et al., 2020).

It is not always clear if or to what extent differences in prey detection and foraging behavior translate to differences in diet. Insectivores with different foraging behaviors may still target the same prey (e.g., Brigham and Fenton 1991; Kent and Sherry 2020). Prey movement may also overlap with the foraging range of more than one type of predator. Still, foraging behaviors and prey detection methods that do correspond to dietary differences may decrease interspecific competition among NAIs.

To our knowledge, this is the first study to use fecal DNA barcoding to investigate the diets of multiple, distantly related, co-occurring NAIs. Our objectives were two-fold. First, we developed a reference barcode database from 56,191 locally collected arthropod specimens to provide more accurate taxonomic assignments of potential prey items than possible in previous studies. We then used DNA barcoding of fecal samples to match prey DNA to our insect barcodes and determine the degree to which NAI diets differ. We expected that differences in diet would depend on NAI species identity and correspond with 1. prey detection methods (i.e., echolocation or visual hunting) and 2. differences in foraging behavior (i.e., aerial hawking or sit-and-wait predators). We found evidence of dietary partitioning among all species. Additionally, eared moths

were consumed significantly more often by birds than by echolocating bats, suggesting that evolutionary interactions between bats and moths may enhance resource partitioning between bats and birds.

MATERIALS AND METHODS

Study Area

The study area encompassed c. 3500 hectares of conservation property in western Montana (www.mpgranch.com; 46°41' N, 114°00' W). Historic management practices include cattle grazing, logging, and agriculture. Current conservation strategies include restoring native grasslands and shrublands, primarily through weed control, seeding and planting efforts, wildlife management, and irrigation. Sampling occurred in mid-elevation forest/grassland, mid-elevation forest, floodplain forest, mid-elevation sagebrush, mid-elevation sagebrush/woodland, and mid-elevation shrubland/grassland plant communities. Elevation ranged from approximately 970 meters in floodplain areas to around 1650 m in higher elevation forests.

Sample collection and processing

We collected fecal samples from NAIs May through September during 2017 and 2018. We captured bats monthly after evening emergence in mist nets set over dry land, streams, and ponds. We placed bats in individual paper bags to collect their fecal pellets. Six additional bat species occur in the study area but were excluded from this study due to low sample sizes. We collected fresh fecal samples from Flammulated Owls, Common Poorwills, and Common Nighthawks captured in mist nets on or near breeding territories. Common Nighthawks and Common Poorwills were also sampled opportunistically near roads or at known nest or roost sites via hand nets. We placed all fecal samples in vials containing ethanol in the field and stored them in a freezer at -20° C until further processing. We labeled samples by the plant community in which they were collected, though sampling location does not always equate to plant community used while foraging. A subsample of Common Nighthawks, Flammulated Owls, and Common Poorwills were also fitted with GPS tracking devices to gather data on home and foraging ranges. Telemetry data for Common Nighthawks indicated foraging ranges up to 400 ha, whereas Common Poorwills ranged between 0.5 and 3.0 ha and Flammulated Owls generally foraged in <1.0 ha (Supplementary Table 1). The daily foraging range of bats sampled varies between < 1 km for Long-eared Myotis to > 4.4 km for Big Brown Bats (Maxell, 2015). Based on observational and telemetry data, home and foraging ranges overlapped for all species.

The Canadian Centre for DNA Barcoding (CCDB) performed all DNA extractions, amplification, and sequencing. DNA extraction and PCR amplification followed CCDB protocols as described in Moran et al., (2019). Samples were incubated overnight in a lysis buffer, concentrated and dried by centrifugation, and finally eluted using a Tris-HCl elution buffer. The CCDB also processed negative controls in parallel with samples to ensure that contamination did not occur. The cytochrome C oxidase 1 (CO1) region was amplified from each sample using the arthropod-specific primers, ZBJ-ArtF1c_t1 and ZBJ-ArtR2_t1 (Zeale et al., 2011), as described previously (Moran et al., 2019; Prosser & Hebert, 2017). Following amplification, samples were pooled and purified. The CCDB performed sequencing on an Ion Torrent PGM following standard protocols (Prosser & Hebert, 2017).

Constructing a DNA barcode library from local Arthropoda

In 2017 and 2018, we collected nocturnal insects monthly May-August using mercury vapor and black lights placed in front of a white sheet and an aerial flight-intercept trap at sites across our study area. In 2019, we expanded insect sampling to include bulk samples collected weekly over 13 weeks (May-August) from flight-intercept, pitfall, and yellow and blue pan traps. We sent samples to the CCDB for sequencing and

identification (deWaard et al., 2019; Ratnasingham & Hebert, 2007). Technicians at CCDB counted total insect abundance by order, weighed biomass, and collected tissue samples from several representatives of each morphospecies. All records are publicly available on BOLD under the datasets MPG and MPGR with photos of specimens to aid in future identification. The resulting local arthropod DNA barcode library consisted of 56,191 Arthropoda specimens collected May-September from 2017 to 2019 at 48 sites within our study area. Nearly all (99.5%) of the specimen sequences were assigned to order, 92.8% to family, 58.0% to genus, and 24.4% to species. A total of 52,033 of the sequences gained Barcode Index Numbers (BINs) in the Barcode of Life Database (BOLD), comprising 6,080 total unique BINs. This effort added 1,529 previously undocumented arthropod records to BOLD, and represented 38 orders, 383 families, 1,810 genera, and 1,740 total species. Dominant orders represented in the final database included Diptera, Hymenoptera, Hemiptera, Lepidoptera, and Coleoptera (Supplementary Figure 1).

Data analysis

We processed demultiplexed sequences using QIIME2 version 2020.2 (Bolyen et al., 2018). We removed all primers prior to analysis using the cutadapt plugin (Martin, 2011) and denoised sequences using the DADA2 denoise-pyro plugin (Callahan et al., 2016). DADA2 is sensitive to single base-pair differences among sequences and produces unique "amplicon sequence variants" (ASVs). The median base pair quality score for all sequences was maintained above 25. Denoised sequences shorter than 100 bp were removed from analyses. We clustered sequences into operational taxonomic units (OTUs) based on a 97% sequence similarity threshold (Vamos et al., 2017), using the VSEARCH plugin (Rognes et al., 2016). We removed sequences only occurring in a single sample or that were represented by fewer than 0.001% of sequences to limit artifactual sequences.

We determined taxonomic assignments using our local DNA barcode library and the BLAST plugin within QIIME2, with a coverage value of 0.7 and sequential percent matching identities of 100, 99, 98, and 97%. If taxonomy could not be assigned to our local database using these parameters, we used a global COI database compiled from BOLD and GenBank and a pretrained RDP classifier (Porter & Hajibabaei, 2018; Wang et al., 2007). The BOLD accession ID associated with each taxonomic identification is indicated where available. We verified all taxonomic identifications based on the plausibility that they may occur within or nearby the study area. All sequences not matching to Arthropoda using either the global COI database or the local database were removed from further analyses. We rarefied samples at 500 sequences, which was sufficient to adequately characterize the species within each sample (Supplementary Figure 2). In total, 77% of OTUs recovered from NAI fecal samples matched 97% or greater with locally collected specimens, while 23% were assigned taxonomy using the RDP classifier.

All statistical analyses were conducted in RStudio Version 1.1.453 using R version 3.6.0, (R Core Team, 2018). We used linear regression to assess differences in diversity metrics based on species, plant community, and collection month. We used a two-way Anova (car package, Fox and Weisberg, 2011) with a type II sum of squares for unbalanced data to test significance of diversity metrics. Where significant, the emmeans package was used for pairwise analyses of diversity metrics between each species (Lenth et al., 2021). To determine insect taxa significantly associated with different NAI species or foraging strategies (i.e., aerial hawking vs. sit-and-wait strategies, or echolocation vs. visual hunting), we used the indicspecies package (Cáceres & Legendre, 2009).

To determine resource partitioning among NAI diet composition, we analyzed both relative read abundance and presence/absence data. Presence/absence data are considered a more conservative option in insectivore fecal analyses (Jusino et al., 2019). However, presence/absence data can also overestimate the importance of prey consumed in small quantities, and it is generally thought that relative read abundances provide more accurate population-level data (Deagle et al., 2019). Even so, we chose to analyze both relative read abundance and presence/absence data and found similar results. We performed all compositional compa-

risons on either Bray-Curtis distances of Hellinger transformed relative read abundances, or Raup-Crick transformed presence/absence data using the vegan package (Oksanen et al., 2019). We assessed differences in diet dispersion (distance from the mean) among species, as significant differences in group dispersions can lead to false positives when testing differences among groups. Because we saw no differences in data dispersion, we used the Permanova function to assess differences in NAI diet composition at the OTU level. For pairwise compositional analyses, we used the pairwiseAdonis2 function in the 'pairwiseAdonis' package (Arbizu, 2017/2021), and adjusted for multiple comparisons (Benjamini & Hochberg, 1995). For each NAI, we also calculated diet turnover among samples using the vegetarian package (Charney & Record, 2012). Diet turnover within each NAI species was calculated based on Shannon beta diversity where zero equals no difference between samples and one represents completely different samples.

RESULTS

Dietary partitioning corresponds with predation of eared insects

Moth families that contain species with ears (Miller & Surlykke, 2001) were associated with the diets of NAIs that hunt visually but not bats that use echolocation or gleaning (p <0.01; Figure 1a; Table 2). The most abundant eared family, Noctuidae moths, occurred in the diets of 82% of Common Poorwills, 69% of Flammulated Owls, and 32% of Common Nighthawks sampled. Long-legged Myotis fecal samples contained Noctuidae moths 35% of the time. However, just 16% of Long-eared Myotis, 4% of Big Brown Bats, and no Silver-haired Bats consumed Noctuid moths. Other eared moth families, including Geometridae, Sphingidae, and Erebidae, also occurred significantly more often in visual hunters' diets, but rarely in bat diets. One other family of eared moths, Drepanidae, associated with the diets of Flammulated Owls and Common Poorwills (p < 0.05, sit-and-wait predators, Table S2), but not Common Nighthawks.

Conversely, the non-eared moth family Tortricidae (mostly spruce budworm), was the most abundant family consumed more often by echolocators than by visual hunters (p < 0.001). We found Tortricidae in the diets of 65% of Long-legged Myotis, 63% of Long-Eared Myotis, 48% of Big Brown Bats, 23% of Silver-haired Bats, but only 11% of Common Nighthawks, 5% of Common Poorwills, and 31% of Flammulated Owls. Ten other insect families were consumed more often by predators that use echolocation (Table 2, p < 0.02). Insect families that were recovered more often in diets of NAIs that hunt by aerial hawking (bats and nighthawks) corresponded with those associated with echolocation strategies (bats only, Table S2). All insect families and OTUs consumed significantly more often by each individual NAI species can be found in Tables S3 and S4.

Dietary Partitioning among species

In general, we saw low dietary overlap among species (Table 3) regardless of foraging behavior or prey detection method. The highest OTU overlap in diets occurred among bats, with the two smallest species, Long-legged Myotis and Western Long-eared Myotis overlapping the most (22%). Big Brown Bat diets also overlapped considerably with all other bat species (18-21%). Among nocturnal birds, the highest overlap occurred between sit-and-wait predators, Common Poorwills, and Flammulated Owls (17%). Diet overlap between bats and birds was the lowest, with Flammulated Owls and Silver-haired Bats overlapping by just 2%.

Controlling for differences between years, Permanova analyses on presence/absence data indicated that NAI species identity explained the largest variation in diet ($R^2 = 0.23$, p = 0.001). Pairwise comparisons indicated that the diets of all NAIs differed from each other (p < 0.006), except for Big Brown Bats, Long-eared Myotis, and Long-legged Myotis, which had more similar diets (Figure 1d; Table S6). Diet composition also varied due to whether NAIs detect prey visually or with echolocation ($R^2 = 0.12$, p = 0.001) and whether they use aerial hawking or sit-and-wait strategies ($R^2 = 0.12$, p = 0.001). Because species was the best predictor of diet, we included it in the full model with collection month ($R^2 = 0.18$, p = 0.001) and plant community ($R^2 = 0.05$, p = 0.001), which were also influential as main effects. The interaction between species and

month was a strong predictor of composition ($R^2 = 0.17$, p = 0.001), highlighting the importance of seasonal variation within each NAI diet. The total variation among insectivore diets explained by the model was 70%. We also performed Permanova analyses on Bray-Curtis distances of compositional data. Relationships were similar, with species ($R^2 = 0.10$, p = 0.001), month ($R^2 = 0.09$, p = 0.001) and their interaction ($R^2 = 0.14$, p = 0.001) explaining the most variation among NAI diets (Table S3). Overall, the model using compositional data explained 55% of the total variation among NAI diets.

At the order level, Common Nighthawks and Long-legged Myotis consumed mostly Diptera (true flies, 75% and 43% of diets, respectively). Common Poorwills and Flammulated Owls consumed mostly Lepidoptera (moths/butterflies; 63% and 88%, respectively). Silver-haired bats were the only NAI to mostly consume Ephemeroptera (mayflies, 45%). In comparison, Big Brown Bats and Western Long-eared Myotis consumed similar abundances of Lepidoptera (30% and 44%, respectively), and Diptera (31% and 43%, respectively). The top insect orders consumed by all NAI species combined were Lepidoptera followed by Diptera, Ephemeroptera, and Coleoptera (Figure 1b). Except for Long-legged Myotis, the top insect families consumed for each NAI were consistent between 2017 and 2018 (Figure 2).

We found craneflies belonging to the genus *Tipula* in 35% of samples overall, more frequently than any other insect genera recovered. The most abundant and common prey OTU matched 100% with the family Tipulidae (BOLD:ADC2461, crane flies, Table 4). Morphological examination of specimens associated with this OTU confirmed it as *Tipula (Lunatipula) splendens* Doane 1901 (personal communication, Dr. Jon K. Gelhaus, June 26th, 2021). This OTU occurred in 26% of all samples, in all NAI species diets except for Flammulated Owls, and was one of the most frequent *and* abundant prey items consumed by Common Poorwills, Common Nighthawks, Big Brown Bats, Long-eared Myotis, and Long-legged Myotis (Table 3).

The second most abundant OTU matched locally to *Choristoneura freemani* (western spruce budworm; BOLD:ABX5883) and was detected in 16% of all NAI samples. It was one of the top two OTUs consumed by most bat species but occurred in just one Common Nighthawk sample and two Common Poorwill samples.

Dietary breadth and turnover

From the fecal samples of all seven NAI species, we identified 73 arthropod families, 165 genera, and 382 OTUs. Silver-haired bats had the widest diet breadth at the order (10) and family (36) levels (Table S7), whereas Common Poorwills consumed the greatest number of insect genera (75) and OTUs (154). We detected the fewest total OTUs in Common Nighthawk samples (50). Flammulated Owls and Common Poorwills had the highest variation or turnover among samples, whereas Silver-haired Bats and Western Long-eared Myotis had the lowest (Table 1). Long-legged Myotis had the most OTU-rich diet on average (Figure 1c), consuming more prey OTUs than Common Nighthawks, Big Brown Bats, and Long-eared Myotis (p < 0.001; Table S8). NAI species, collection month, year, and plant community were significant predictors of dietary richness. Species identity had the greatest influence.

DISCUSSION

Eared moths are eaten more often by nocturnal birds than bats

In this study, we observed previously unreported dietary partitioning among co-occurring nocturnal aerial insectivorous birds and bats. Variation in NAI diets correlated with prey detection method based on both presence/absence and compositional data. This trend was driven mainly by birds successfully preying on eared moths more often than bats. Previous studies of bat diets predominately used visual examinations of feces, which inhibits investigations of moth's auditory abilities and often resulted in order level taxonomic designations of prey. As such, this may be the first evidence that multiple families of eared moths largely avoid predation by a suite of bat species in their natural environment.

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Eared moths can detect bat echolocation calls from farther away than bats can detect moths, approximately ten times farther in the case of Noctuid moths (Surlykke et al., 1999). As a result, moth adaptations to avoid bats (Hofstede & Ratcliffe, 2016; Waters, 2003) leave open niche space for nocturnal insectivorous birds that hunt visually. Complementary to visual detection methods, both Common Poorwills and Common Nighthawks have a velvety coating on wing and tail feathers adapted for quiet flight (Clark, LePiane, and Liu 2020), which may make them difficult for eared moths to detect. Indeed, Eared moths, especially Noctuid moths, made up a large portion of Common Poorwill and Common Nighthawk diets, demonstrating the success of quiet flight adaptations. Flammulated Owls also fly quietly and possess relatively long wings that allow them to move quickly (though perhaps without much agility) throughout the forest canopy (Johnson, 1997). Rather than aerial hawking, Flammulated Owls, like Common Poorwills, primarily use a sit-and-wait hunting strategy. This consists of flying from a perch inside the tree crown to capture insects resting in other areas of the same crown or adjacent trees (Reynolds & Linkhart, 1987). Together, these results indicate that birds that can ambush prey, rather than alert them with echolocation calls, can initiate successful attacks on eared insects at closer ranges.

The lower occurrence of eared moths in bat diets demonstrates the effectiveness of moth adaptations to bat predation (Hofstede & Ratcliffe, 2016). Still, Long-legged and Long-eared Myotis tended to consume eared moths at slightly higher rates than the other bats in this study. Long-legged Myotis makes echolocation calls at higher frequencies and detects prey at greater distances than Big Brown Bats and other myotis species, which may give it an advantage (Fenton & Bell, 1979; Saunders & Barclay, 1992). Alternatively, Long-eared Myotis uses passive hearing and low-amplitude calls while gleaning, which are undetectable by some eared moths (Faure, Fullard, and Barclay 1990). Gleaning by Myotis species evolved subsequent to echolocation strategies (Morales et al., 2019) and may be a counteradaptation to reduce detection by eared prey (Razak, 2018). However, gleaning may also have evolved as a general adaptation to hunting in cluttered areas (Brinkløv et al., 2010). An obvious counterstrategy to eared prey would be for bats to use a sit-and-wait hunting strategy. However, the physiology of most bats precludes them from leaping into flight (Schutt et al., 1997).

The decreased ability of bats to capture eared moths may result in more specialized diets compared to nocturnal birds. For example, Common Poorwills and Flammulated Owls had greater diet variation among individuals than other NAI species. This, together with their small foraging ranges, may indicate that they are relatively generalist consumers. Although these birds consumed a larger proportion of eared moths, they may opportunistically sally after any large insect they see from their perch. Previous investigations found that Common Poorwills only consumed prey >5mm in length, despite a higher abundance of smaller insects, potentially due to visual constraints (Bayne & Brigham, 1995). We did not find any evidence contradicting this, however since we used DNA instead of morphology to indicate prey, we were unable to definitively determine prey size.

In addition to moths, ultrasonic hearing via tympanal organs has evolved independently within at least eight other insect orders, including Orthoptera, Mantodea, Blattodea, Hemiptera, Hymenoptera, Coleoptera, Neuroptera, and Diptera (Göpfert & Hennig, 2016; Hoy & Robert, 1996). Besides serving to detect and avoid predators, insect hearing has also evolved as a means of communication (Hoy & Robert, 1996). In Neuroptera, green lacewings can detect ultrasonic frequencies and avoid predation by bats (Miller, 1975), and a recent study indicates a similar ability in Myrmeleontidae of the Neuroptera (antlions) (Holderied et al., 2018). However, no insect family with known tympanal hearing abilities were significantly associated with bat diets in this study. Other insect families have evolved different mechanisms of hearing (e.g. Culicidae; Hoy & Robert, 1996), however these insects did not appear to avoid detection by bats more than birds.

Non-eared prey partitioning among bats and birds

Though these results show a clear link between the ultrasonic hearing of moths and their absence in bat diets compared to nocturnal birds, the partitioning of non-eared insects is less clear. Moths in the family Tortricidae lack hearing organs (Fullard & Napoleone, 2001). This may explain why bats consumed Tortricidae in such high amounts. The most commonly consumed Tortricidae moths, spruce budworm, tend to fly near treetops

(Soutar & Fullard, 2004). Common Nighthawks that hunt high above the ground and Flammulated Owls that hawk from tree perches would still encounter these insects. Indeed, 11% of nighthawks and 31% of Flammulated Owls consumed Tortricidae in this study. However, for Common Poorwills that generally hunt only up to three meters above ground (Brigham and Barclay 1992), spruce budworm may often be out of range. This would explain why Common Poorwills preyed on Tortricidae moths less often than all the other NAIs.

Previous studies suggest that variation in echolocation calls leads sympatric bat species to detect different prey resources, enabling coexistence (Razgour et al., 2011). However, such diet partitioning has not been shown empirically among the assemblage of bats in our study. Although overall diet composition did not differ significantly among Big Brown Bats, Long-legged Myotis and Long-eared Myotis (Permanova), we observed low overlap in the insect taxa consumed (18-22%), suggesting some specialization. This pattern indicates that although these bats consume high abundances of the same species (i.e. spruce budworm), coexistence may be promoted due to differences in species consumed at lower frequencies. This hypothesis was also supported by stronger differences among species when analyzing presence/absence data compared to relative abundances, which is less sensitive to rare species. Additionally, minor differences in foraging locations may enable coexistence among sympatric species with similar foraging behaviors (Kent & Sherry, 2020), or resources like spruce budworm may be abundant enough to render partitioning unnecessary. Still, greater sampling efforts in the future may reveal finer-scale diet partitioning among the bats in this study that we were unable to detect here.

Conservation implications

North American avifauna have decreased in abundance by approximately 29% since 1970 (Rosenberg et al., 2019). Aerial insectivores are even more threatened (Nebel et al., 2010; Spiller & Dettmers, 2019). Bats face conservation threats globally and regionally (Frick et al., 2020). Though many factors contribute to declining population trends, decreases or changes in food availability play a role, making identification of key food sources important (Rosenberg et al., 2019; Spiller & Dettmers, 2019). The 73 arthropod families, 165 genera, and 382 OTUs identified in NAI diets in our study far exceed previous documentation, particularly at high taxonomic resolution, for most NAI species. However, there is still much work to be done with resolving the different taxa in the NAI diets. Tipulidae (crane flies) especially, were often not resolved beyond the family level here, yet were the most common order found in the diet of 4 of the 7 NAIs.

Crane flies constitute the majority of prey for various wildlife, including snails, salamanders, other Arthropoda (Lunghi et al., 2020), in addition to the NAIs observed here. A recent study found that crane fly abundance was a key predictor of the persistence of multiple sympatric bird species, and explained 39% of observed bird abundance (Carroll et al., 2015). This suggests that any decline in crane fly populations may be paired with future declines in avian populations. Monitoring crane fly populations may help identify high conservation priority areas as these insects are susceptible to plant community degradation and loss (Yadamsuren et al., 2015) and changes in water quality (Morse et al., 1994). Crane fly larvae in particular, are susceptible to desiccation (Pritchard, 1983), and prolonged drought or extreme heat caused by future climate change may harm crane fly populations (Carroll et al., 2011). The importance of crane flies in NAI diets highlights the need for expanded analyses on crane fly ecology and conservation, especially as many species have yet to be described (Marshall, 2012).

Knowledge of NAI diets can also identify regulators of unwanted pests such as western spruce budworm, cutworm moths, and Douglas fir tussock moths that cause crop and forest damage. Western spruce budworm in particular, is a common conifer defoliator that reduces tree growth in the Pacific Northwest (Fierravanti et al., 2019). Because NAIs consume pests like spruce budworm in high and variable proportions, future research into the possible cascading effects on forest biomass and soil carbon retention may have global implications (Schmitz et al., 2017). Overall, our findings indicate that the evolutionary interactions between bats and moths may promote the coexistence of multi-phyla predator communities. Future management practices

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that promote both eared and non-eared prey insects may add stability to already threatened insectivore populations.

ACKNOWLEDGEMENTS

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TABLES

Table 1. Characteristics of nocturnal insectivores included in diet analyses . Sample number, average mass of local specimens, prey detection methods, foraging behavior, and diet turnover (diet variation among individuals) of the seven nocturnal aerial insectivores sampled for dietary analyses.

Table 2. Results from indicator prey analysis.

Insect families significantly associated with specific prey detection strategies or foraging behaviors (p<0.05)

Table 3. Diet overlap among co-occurring insectivores based on insect OTUs recovered from fecal samples.

Table 4. Top prey consumed by each insectivore species. Most frequent and abundant insect OTUs consumed by each nocturnal aerial insectivore.

Figures

Figure 1. Variation in composition and richness of insectivore diets. (a) The percent relative sequence abundance of arthropod families found in the diets of seven nocturnal aerial insectivores. The size of points indicates the percent relative sequence abundance within each species and red outline indicates arthropod families significantly associated with the diet of an individual insectivore. Asterisks indicate moth families with tympanal ears. The grey bars in (b) indicate the relative sequence abundance of insect families in the diets of all insectivores combined. Only insect families that represented > 5.0% of any insectivore diet are shown. Diet richness (c) and principal coordinate analysis (PCoA = Multidimensional scaling) of diet composition of the seven sympatric nocturnal aerial insectivores (d) are based on OTUs of arthropod prey. Compositional means are represented by points, and error bars represent standard error for each insectivore species diet. Ellipses are overlayed to indicate variation between echolocating and visual predators.

Figure 2. Diet variation between sampling years. Comparison of the relative abundances of insect families consumed by co-occurring nocturnal aerial insectivores over a two-year period. Only families represented by > 5.0% of total relative sequence abundance for an insectivore are shown.

Figure 1.
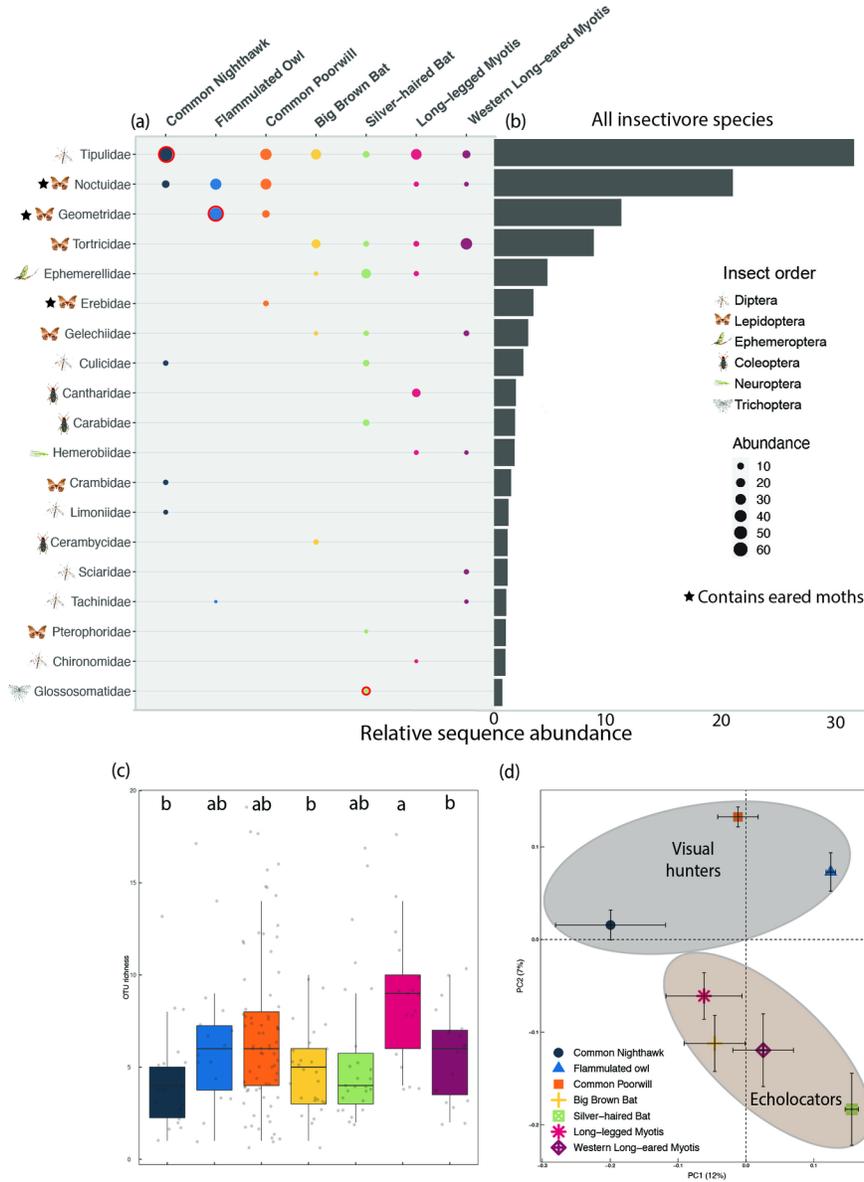
Figure 2.

Hosted file

image6.emf available at https://authorea.com/users/328742/articles/710872-do-the-evolutionary-interactions-between-moths-and-bats-promote-niche-partitioning-between-bats-and-birds