

Prey selection by *Chordeiles minor* (Common Nighthawk) does not reflect differences in prey availability between breeding and nonbreeding grounds

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ABSTRACT

Predators may adjust their diets to match their energy needs and food availability, but these adjustments have not been explored for migratory aerial insectivores outside of the breeding grounds. We found that *Chordeiles minor* (Common Nighthawk), a long-distance migrant and member of the rapidly declining aerial insectivore guild, exhibited similar levels of diet richness, diet diversity, and prey selectivity on the breeding and nonbreeding grounds, despite large differences in prey abundance. We examined the diets and prey communities of *C. minor* during 2 breeding seasons in Florida, USA, and 2 nonbreeding seasons in Corrientes Province, Argentina (2020–2022). We used DNA metabarcoding to identify insect prey in *C. minor* fecal samples, and we employed malaise and UV light traps to assess abundance and composition of aerial insect prey communities. Abundance and richness of available prey were significantly higher on the nonbreeding grounds than on the breeding grounds. Even so, *C. minor* exhibited similar within-sample and within-population diet richness, Shannon and Simpson diversities, and prey preferences at both sites. Adults differed in their consumption of Lepidoptera between sites: adults on the nonbreeding grounds preferred Lepidoptera over all other orders, whereas adults on the breeding grounds consumed Lepidoptera less frequently than expected. We suggest that breeding adult *C. minor* may deliver Lepidoptera to their young instead of consuming this prey. At both sites, *C. minor* showed preference for large-bodied, nutrient-rich prey, suggesting that these generalist predators exhibit less diet flexibility than previously thought and thus may be vulnerable to changes in prey communities at multiple points in the annual cycle.

Keywords: aerial insectivore, annual cycle, *Chordeiles minor*, Common Nighthawk, diet, DNA metabarcoding, prey selection

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LAY SUMMARY

- Predators that adjust their diets to match energy needs and prey abundances may be less vulnerable to changes in prey availability than predators lacking dietary flexibility.
- We used DNA metabarcoding and aerial insect sampling to evaluate the diet diversity and prey selection of *Chordeiles minor* (Common Nighthawk) on their breeding grounds in the United States and nonbreeding grounds in Argentina.
- The abundance, richness, and diversity of available prey differed between sites, but *C. minor* diet diversity and richness did not differ.
- Diets were dominated by Hemiptera and Hymenoptera, which were consumed more than expected by their relative availability.
- *Chordeiles minor* consumed Lepidoptera more than expected on nonbreeding grounds, but less than expected on the breeding grounds, where they may have chosen to give Lepidoptera to their young.
- Our results suggest that *C. minor* could be vulnerable to changes in insect communities on both the breeding and nonbreeding grounds.

La selección de presas por *Chordeiles minor* no refleja diferencias en la disponibilidad de presas entre las zonas de cría y las de no cría

RESUMEN

Los depredadores pueden ajustar sus dietas para satisfacer sus necesidades energéticas o disponibilidad de alimentos, pero no se ha explorado estos ajustes en insectívoros aéreos migratorios fuera de sus zonas de reproducción. Encontramos que *C. minor* (el añapero boreal), un migrante de larga distancia y miembro del gremio de insectívoros aéreos en rápido declive, presentó niveles similares de riqueza de la dieta, diversidad de la dieta y selectividad de presas en las zonas reproductivas y las no reproductivas. Examinamos las dietas y las comunidades de presas de *C. minor* durante dos temporadas de cría en Florida, EE.UU., y dos temporadas no reproductivas en la provincia de Corrientes, Argentina (2020–2022). Utilizamos metabarcoding de ADN para identificar insectos en muestras fecales de *C. minor*, y empleamos trampas malaise y de luz UV para evaluar la abundancia y la composición de las comunidades de presas (insectos aéreos). La abundancia y riqueza de presas disponibles fueron significativamente mayores en las zonas no reproductivas que en las reproductivas. Aún así, *C. minor* mostró una riqueza de dieta, diversidades de Shannon y Simpson y preferencias de presas (dentro de cada muestra y dentro de la población) similares entre ambos sitios. Los adultos diferían en su consumo de lepidópteros entre sitios: los adultos de las zonas no reproductivas prefirieron los lepidópteros a todos los demás órdenes, mientras que los adultos de las zonas reproductivas consumieron lepidópteros con menos frecuencia de lo esperado. Sugerimos que los adultos reproductores pueden entregar lepidópteros a sus crías en lugar de consumirlos. En ambas estaciones, *C. minor* mostró preferencia por presas de gran tamaño y ricas en nutrientes, lo que sugiere que estas aves generalistas muestran menos flexibilidad en su dieta de lo que se pensaba y podrían ser, por tanto, vulnerables a los cambios en las comunidades de presas en múltiples etapas del ciclo anual.

Palabras clave: añapero boreal, *Chordeiles minor*, selección de presas, dieta, metabarcoding de ADN, insectívoro aéreo, ciclo anual

INTRODUCTION

Optimal foraging theory predicts that predators should alter their foraging strategies to match changes in their energy needs and prey availability (Pyke 1984). During high-energy periods such as breeding, adults are predicted to increase their efficiency by foraging in higher quality habitats (Geary et al. 2020) or by targeting more energy-rich prey (Jenkins and Jackman 1994). Such adjustments have been observed in birds, including raptors and passerines, and in bats (Törnberg 1997, Naef-Daenzer et al. 2000, Agosta et al. 2003). Understanding the prevalence of this behavior is of particular importance as climate change and habitat loss and degradation may create conditions where species that do not forage optimally become vulnerable to changes in their food supply (McKinney 1997, Clavel et al. 2011, Crowley et al. 2016).

Aerial insectivores have diverse and often flexible diets but also represent one of the most imperiled bird guilds in North America, in part because of broadscale declines in insect populations (Spiller and Dettmers 2019, Sauer et al. 2020). Recent studies indicate that many aerial insectivores exhibit dietary specialization in the form of prey selection and may be more sensitive to changes in prey availability than previously thought (Trevelline et al. 2018, McClenaghan et al. 2019, Wray et al. 2020). Migratory aerial insectivores could be particularly vulnerable because individual birds must obtain prey from a series of different habitats along their annual cycle.

Neotropical–Nearctic migrants spend most of their annual cycles in nonbreeding areas throughout Central and South America and travel to higher latitudes to take advantage of spring and summer food pulses (Somveille et al. 2018). Although food may be abundant in breeding areas, the energetic costs of reproduction are distinct from energetic costs faced during nonbreeding periods (Cucco and Malacarne 1995). Breeders also must allocate a large proportion of captured prey to their young, which can increase the time spent foraging (Schifferli et al. 2014, Hernández-Pliego et al. 2017). Outside of the breeding period, Neotropical–Nearctic migrants spend energy molting and replenishing the fat reserves needed for long-distance migration (Barta et al. 2008, Kelly et al. 2013). While many studies examine prey availability and diets of aerial insectivores during the breeding season, comparable information from the nonbreeding season is scarce. Additionally, most diet studies focus on small-bodied aerial insectivores that forage for long periods, such as swallows and small bats, which may be less affected by changes to prey communities than large-bodied species with constricted foraging windows.

We studied spatiotemporal variation in diet and prey selection in *Chordeiles minor* (Common Nighthawk) across 2 breeding seasons in North America and 2 nonbreeding seasons in South America. *Chordeiles minor* populations, like those of many other aerial insectivores, are in steep decline, with current estimates reporting an average trend of $-1.8\% \text{ yr}^{-1}$ for *C. minor* across the United States and Canada (Sauer et al. 2017). This species occupies large ranges, spanning from northern Canada during the breeding season into central Argentina during the nonbreeding season (Brigham et al. 2020). Breeding season studies in Canada found adult *C. minor* consumed Coleoptera (beetles) and Hymenoptera (ants, wasps, bees, and sawflies) more frequently than expected by their relative availability, whereas they appeared to avoid Diptera (flies, mosquitoes, and midges; Brigham 1990, Brigham and Fenton 1991, Todd et al. 1998). In boreal forests, Coleoptera made up 80% of the biomass of food boluses delivered to nestlings (Knight et al. 2018). Coleoptera and Hymenoptera are common in the diets of other aerial insectivores and provide high proportions of crude fat and protein, whereas Diptera provide fewer nutrients but are easy to digest (Levin et al. 2009, Lease and Wolf 2010, Razeng and Watson 2015). Lepidoptera (moths) are high in nutritional value and common in other nightjar diets (Evens et al. 2020, Mitchell et al. 2022, Souza-Cole et al. 2022), but they were not preferred or avoided by *C. minor* in previous prey selection studies in Canada (Brigham 1990, Brigham and Fenton 1991, Todd et al. 1998). Prey selection for Trichoptera (caddisflies) is inconsistent among breeding season studies, with *C. minor* in one location consuming Trichoptera more than expected (Brigham 1990) and individuals

elsewhere showing no preference (Todd et al. 1998). It is unknown whether *C. minor* exhibit similar preference and avoidance of these orders outside of the northernmost part of the breeding range.

We studied diet diversity and prey selection in *C. minor* that migrate between breeding grounds in Florida, USA (May–August), and nonbreeding grounds in Corrientes Province, Argentina (December–March; Cockle et al. 2023). Our objectives were to (1) identify important prey for *C. minor* during these 2 periods of the annual cycle and (2) evaluate diet flexibility between the breeding and nonbreeding season. We hypothesized that *C. minor* at both sites would favor large-bodied prey with high crude fat and protein contents over less energetically valuable, small-bodied prey. Accordingly, we predicted that prey selection models would show higher relative abundance of Hymenoptera, Lepidoptera, and Coleoptera in *C. minor* fecal samples, and lower relative abundance of Diptera, than expected based on each order's relative abundance in the environment. We also hypothesized that *C. minor* diets would shift to become more selective on the breeding grounds than on the nonbreeding grounds because of the high energetic requirements associated with reproduction and brood rearing. Under this hypothesis, we predicted that *C. minor* diets would be less diverse, and prey selection models would show preference for fewer orders, on the breeding grounds than on the nonbreeding grounds.

METHODS

Study Areas

Citrus Wildlife Management Area (hereafter Citrus) is a 200 km² tract in central Florida dominated by native longleaf pine (*Pinus palustris*) sandhills (28.778°N, 82.406°W). Prescribed burns are conducted every 2 to 5 years within ~260 ha plots, creating a mosaic of burned and unburned forested areas with herbaceous understories and patches of bare ground where *C. minor* nest. An adult female GPS-tagged at Reserva Natural Rincón de Santa María (see below) spent a breeding season at Citrus and appeared to have 2 nests, each in a plot burned <6 months prior (Cockle et al. 2023). *Chordeiles minor* are present at Citrus from mid-April until late September, although the earliest and latest individuals may breed elsewhere (eBird 2024). We found early nests in the first week of June and nestlings with natal down as late as 21 July.

Reserva Natural Rincón de Santa María (hereafter Santa María) is a 35 km² provincially protected nature reserve that sits on the southern bank of the Yacyretá Reservoir in Corrientes, Argentina (27.530°S, 56.600°W). Current vegetation communities on the reserve feature native grasslands, freshwater marsh, scattered remnants of secondary riparian forests, and stands of exotic eucalyptus (*Eucalyptus* spp.) and pine (*Pinus* spp.; Bauni and Schivo 2015). Santa María is an important site for the conservation of nightjars (family Caprimulgidae) and hosts 9 nightjar species, including *C. minor* in December through March. *Chordeiles minor* principally forage high above a mosaic of grasslands and stands of pine and eucalyptus.

Prey Availability

We quantified prey availability by sampling aerial insect communities at dusk at both study areas. *Chordeiles minor* are crepuscular and have short foraging windows (<2 hr) around dawn and dusk (Cockle et al. 2023). Similar to other studies of aerial insectivores, we used Lightweight Malaise Traps (Bioquip Products, Rancho Dominguez, CA) to intercept diurnal and nocturnal insects that fly close to the ground (e.g., Diptera, Trichoptera, Blattodea, Orthoptera, and Hymenoptera). We used UV light traps with 20-watt bulbs to draw in nocturnal insects that fly up to 30 m above ground and are phototactic, or attracted to light (e.g., Coleoptera, Lepidoptera, and Odonata; van Grunsven et al. 2014, Montgomery et al. 2021). Malaise traps and UV light traps were deployed >10 m apart to minimize interference between traps. Traps were opened 1 hr before sunset and closed 3 hr later. Traps contained 95% ethanol to kill and preserve insects prior to processing.

We rotated insect traps among areas on both study sites where *C. minor* were observed foraging. Traps at Citrus were placed at 10 points along 27 km of dirt road that spanned the 20,000-ha study area. Traps at Santa María were placed at 11 points along a 5-km stretch of the southern edge of the reservoir. In the first year (hereafter 2021), we sampled each point once (from 8 December 2020 to 25 February 2021 at Santa María and from 7 June to 3 August 2021 at Citrus). In the second year (hereafter 2022), we sampled all points twice (from 6 December 2021 to 16 February 2022 at Santa María, and from 24 May to 27 July 2022 at Citrus). Insect sampling coincided with periods when *C. minor* were present at each site. We identified insects to order and measured body lengths to the nearest 0.1 mm. For highly abundant morphospecies (i.e., species differentiated by distinctive morphology), we measured 5 individuals each and applied the mean length to other individuals. We ran all analyses separately for Malaise and UV traps.

***Chordeiles minor* Diet**

We collected fecal samples from *C. minor* captured at Citrus (7 June to 6 August 2021 and 26 May to 27 July 2022) and Santa María (4 December 2020 to 5 March 2021 and 7 December 2021 to 15 February 2022). At Citrus, we primarily captured adults with mist nets and audio lures but also captured incubating or brooding females with a spotlight and a handheld net. We set up mist nets and playback devices ~20 min after sunset and ran them until all *C. minor* activity stopped (typically ~45 min after sunset). At Santa María, where individuals regularly rest on dirt roads and rocky platforms after dark, we searched for *C. minor* by driving the reserve's circuit of roads for ~2 hr beginning at dusk, and we captured individuals with spotlights and a handheld net. We used clean forceps or a popsicle stick to collect a fecal sample directly on cloaca or from inside a bleached cloth bird bag. We classified a sample as "clean" if it was collected from the cloaca or bird bag, and "unclean" if the sample fell and was collected from another surface. Samples were stored in 95% ethanol at 4°C. Multiple samples collected from the same individual were sequenced separately in case sequencing failed.

We extracted prey DNA from fecal samples following Snider et al. (2022), with the exception that we dried samples in a fume hood overnight to ensure all ethanol evaporated. We then immersed the sample in lysis buffer with 0.1- and 0.5-mm zirconia-silica beads and homogenized the mixture in a Mini-BeadBeater 24 (BioSpec Products, Bartlesville, OK, USA) to break down insect exoskeletons and cell membranes. We isolated DNA using SPRI beads and a series of ethanol washes before eluting DNA with 10 mM Tris-HCl. We quantified DNA in each sample using a Denovix spectrometer and dsDNA High Sensitivity Assay kit (Denovix, Wilmington, DE, USA), concentrating any samples $<0.2 \text{ ng } \mu\text{L}^{-1}$ in a vacuum centrifuge. We completed extractions in sets of 6–16 samples, and each set included an extraction negative with no fecal material.

Library preparation included 2 rounds of polymerase chain reaction (PCR) on a Mastercycler ProS (Eppendorf North America, Enfield, CT, USA): (1) amplification of a target sequence (PCR1); and (2) annealing of indexes (PCR2). For PCR1, we amplified a 180 base pairs (bp) section of the cytochrome-c oxidase subunit 1 (COI) gene using the ANML universal primer set (Jusino et al. 2019), which targets a wide range of arthropods commonly found in *C. minor* diets (Wray et al. 2020). We added Illumina overhangs to primers to allow for annealing of indexes during post-PCR library preparation (Illumina 2013) and performed PCR1 in triplicates (Vo and Jedlicka 2014, Alberdi et al. 2019). We included extraction negatives in PCR1 and included a PCR negative in each plate (molecular-grade water instead of DNA). Reagent concentrations and thermocycler conditions followed Jusino et al. (2019; Supplementary Material S.1). We visualized a subset of PCR1 product on a 1.2% agarose gel to ensure successful amplification before combining all triplicates. We then cleaned the pooled PCR1 product with SPRI beads at a 1.2x bead:PCR product concentration to isolate the amplicon of interest. Finally, we confirmed successful cleanup by visualizing a subset of PCR1 products on agarose.

In PCR2 (index annealing), we used the Nextera XT Index Kit v2 and followed the Illumina 16S

Metagenomic Sequencing Library Preparation protocol (Illumina 2013) for paired-end sequencing (see Supplementary Material S.2 for details). After visualizing a subset of PCR2 products to confirm that indexes annealed properly, we cleaned the PCR2 product with SPRI beads at a 0.9x bead:PCR product concentration to remove residual adapter dimer.

We normalized the cleaned libraries to 4 nM by combining calculated volumes of cleaned PCR2 product and Tris-HCl. We used a vacuum centrifuge to concentrate any samples <4 nM to a final volume of 5 µL. Finally, we combined 5 µL of each normalized library to create a single pooled library that was sent to Pennington Biomedical Research Center (Baton Rouge, LA, USA) for sequencing. The pooled library was quantified on an Agilent Bioanalyzer, spiked with 15% PhiX (Alberdi et al. 2017, Trevelline et al. 2018), run on an Illumina MiSeq platform with v2 reagent kit (Illumina 2013) using paired-end sequencing, and demultiplexed at the facility. Raw sequences can be found at <https://www.ncbi.nlm.nih.gov/sra>, BioProject: PRJNA1120945, Accession Numbers SAMN41721490–SAMN41721561 (Citrus) and SAMN41739139–SAMN41739234 (Santa María).

Custom Reference Library

We developed a reference library to assign taxonomic identities to barcode sequences from Santa María because public reference libraries such as the Barcode of Life Database (BOLD; <https://boldsystems.org/>) and the National Center for Biotechnology Information (NCBI; <https://blast.ncbi.nlm.nih.gov/Blast.cgi>) lacked entries for many insects found in northern Argentina. We selected 175 of our most commonly captured insects and identified 129 to genus, 3 to family, and 43 to order. We then extracted DNA from 1–4 legs of a voucher specimen using a Quick-DNA™ Miniprep Plus Kit (Zymo Research, Irvine, CA, USA) and performed PCR to target the COI gene with the same primers and conditions used for fecal PCR1 described above. We cleaned the PCR product with an Exo-SAP protocol (Supplementary Material S.3) and performed cycle sequencing on the cleaned product in both forward and reverse directions (Supplementary Material S.4). Cycle sequencing product was cleaned with Sephadex G50 (Cytiva, Marlborough, MA, USA) then Sanger sequenced on an ABI 3130xl Genetic Analyzer at the Louisiana State University Genomics Facility.

We used Geneious 2022.1 to trim sequences and exclude low-quality reads, and we aligned forward and reverse reads using the consensus method. Voucher insects were deposited in the entomology collection of the Bernardino Rivadavia Museum of Natural Science in Buenos Aires, Argentina. All barcode sequences are publicly available at <http://boldsystems.org>, Sequence Pages CAI001-24–CAI128-24. Barcode sequences longer than 200 bp are also available at <https://www.ncbi.nlm.nih.gov/genbank>, Accession Numbers PQ299157–PQ299179.

Nestling Provisioning

We opportunistically collected food boluses from adult *C. minor* that were captured in mist nets as they attempted to deliver food to nestlings. We collected each bolus dropped upon capture and stored the contents in 95% ethanol at 4°C. We identified insects in food boluses to order.

Statistical Analysis

[LEVEL HEADING 3] Bioinformatics

We imported demultiplexed sequences into Qiime2 2023.5 (Bolyen et al. 2019) and trimmed primers using the *Cutadapt* function (Martin 2011), which also removed reads that did not contain primer sequences. We performed denoising and quality control using *DADA2* (Callahan et al. 2016), which filtered out reads with Phred scores <30, removed chimeras (consensus method), joined paired-end reads, and collapsed reads into amplicon sequence variants (ASVs; Alberdi et al. 2017, Snider et al. 2022). We removed reads that appeared <10 times in total to minimize the chances of including

contaminants or symbiotic, non-target fauna (e.g., mites on aerial insects; Leray and Knowlton 2017). We classified the remaining ASVs using a naïve Bayes classifier, which we trained on all arthropod sequences from NCBI (Robeson II et al. 2020), all Metazoan sequences in BOLD (O'Rourke et al. 2020), and our custom reference library.

We manually reviewed the classified dataset to ensure the taxonomic assignments achieved appropriate confidence levels and resolved any conflicts between reference libraries. We assigned an ASV to species if it matched with > 99% confidence, genus if confidence was between 97% and 99%, family if confidence was between 95% and 97%, and order if confidence was between 90% and 95%. We removed all ASVs with <90% confidence. This approach represents a compromise between less conservative methods used by Jusino et al. (2019) to classify prey and more conservative methods used by Evens et al. (2020). When an ASV matched multiple reference libraries, we selected the entry from the library with the highest confidence. If a taxonomic assignment conflicted between libraries, we selected the lowest common taxonomic assignment. *Chordeiles minor* are not known to eat non-insect taxa (Brigham et al. 2020), so we removed ASVs for Araneae (spiders; $n = 2$), Ixodida (ticks; $n = 1$), and Trombidiformes (mites; $n = 4$). These were likely ectoparasites of consumed insects (Ixodida and Trombidiformes) or the result of sample contamination (Araneae).

[LEVEL HEADING 3] Diet composition and diversity

We present diet composition as both relative read abundance (RRA, an abundance-based metric) and frequency of occurrence (FOO, an incidence-based metric). RRA reflects the proportion of sequences within an individual sample belonging to a given prey item and increasing proportionately with the abundance of the taxon in the diet (Deagle et al. 2019, Verkuil et al. 2022). However, RRA may quantify prey inexactly because not all insects are digested equally, the amount of mitochondrial DNA varies across species, and primers can amplify DNA from some species more readily than others (Elbrecht and Leese 2015, Jusino et al. 2019). We also present FOO, which is the proportion of individual fecal samples containing each prey item. FOO has biases that include giving equal weight to rare and common prey species, ignoring the relative contributions of prey within a single sample, and often requiring more sampling effort to draw conclusions (Cuff et al. 2022). FOO can only be calculated at the population level, while RRA can be calculated at the population level and for individual samples.

To evaluate diet composition, we assessed richness (the total number of unique taxa in a community; does not consider relative abundance) in conjunction with Shannon diversity (a measure of uncertainty about the identity of individuals sampled; considers relative abundance; Shannon 1948), and Simpson diversity (the probability that two sampled individuals are of the same taxon; considers relative abundance; Simpson 1949). We used methods outlined in Hill (1973) to estimate indices (known as Hill numbers) for community richness (q_0), Shannon diversity (q_1), and Simpson diversity (q_2), thus yielding scaled values with magnitudes that researchers can easily compare (Hill 1973, Jost 2006). Hill numbers correspond with but are not identical to the traditional definitions for richness, Shannon diversity, and Simpson diversity given above. Hill indices for richness provide measures of community composition that only consider the number of taxa in an assemblage, while Hill indices for Shannon diversity and Simpson diversity consider relative abundances of taxa present (Alberdi and Gilbert 2019).

We performed all analyses using R 4.3.2 (R Core Team 2023), and considered metrics to be significantly different if 95% confidence intervals (CIs) did not overlap. First, we compared the diversity and richness of clean and unclean fecal samples to assess whether field contamination increased diversity estimates for unclean samples. We used the package *iNEXT.beta3D* (Chao et al. 2023) to calculate Hill numbers for richness, Shannon diversity, and Simpson diversity at the alpha level (within-sample diversity; D_α) and beta level (between-sample diversity; D_β) using abundance-based diet data, and at the gamma level (entire population diversity; D_γ) using both abundance- and incidence-based diet data. We converted raw ASV abundances to RRAs to account for differences in sequencing depth

(McMurdie and Holmes 2014); RRAs represented the proportion of the total reads within a fecal sample that belonged to each insect order. To calculate D_α and D_β , we constructed two matrices (clean and unclean) that contained the RRA of insect orders (rows) detected in fecal samples (columns). We used the function *iNEXTbeta3D* to calculate taxonomic diversity using datatype = abundance, and we increased bootstrap replications from the default (10) to 50 to improve accuracy. To calculate D_γ , we repeated this procedure using both RRA and FOO. For the FOO analysis, we converted all RRAs to presence (1) or absence (0) in each fecal sample, and ran the *iNEXTbeta3D* function with 50 bootstraps and datatype = incidence. We evaluated the output for sample completeness using even coverage, rather than even sample sizes, to rarefy data (Chao et al. 2020, Roswell et al. 2021). This technique required identifying the maximum sample coverage (i.e., the estimated proportion of the true community detected via sampling) that could be attained at Citrus and Santa María by creating a rarefaction curve that was $\leq 2 \times$ the actual sample size collected at each site. Next, we used the sample coverage value of the site with the lowest coverage to obtain diversity estimates and 95% CIs for both sites.

Diversity and richness estimates were higher in clean versus unclean samples (Supplementary Material S.5), suggesting that potential contamination did not lead to higher diversity estimates in unclean samples. Accordingly, we combined clean and unclean samples to calculate final Hill numbers for richness, Shannon diversity, and Simpson diversity. We performed procedures described above to compare D_α , D_β , and D_γ between Citrus and Santa María. We measured similarity in diet richness between sites and years using the Jaccard-type and Sørensen-type indices developed by Chao et al. (2019). To visualize diet richness and overlap between sites and years, we used non-metric multidimensional scaling (NMDS), which plots samples in two-dimensional space based on rank ordered similarity distances (Shepard 1962).

[LEVEL HEADING 3] Prey availability and selection

We calculated the mean and standard error ($\mu \pm SE$) for raw counts of insects captured in Malaise and UV traps and modeled the diversity of prey communities separately for each trap type. We estimated Hill numbers for richness, Shannon and Simpson diversities, Sørensen-type similarity, and Jaccard-type similarity, ensuring equal sample completeness between Citrus and Santa María for each year.

To assess whether *C. minor* consumed prey in proportion to their availability at each site, we modeled prey selection using the R package *econullnetr* (Vaughan et al. 2018). This package uses prey abundance data to create a null model for the expected abundance for each prey item, assuming no prey selection. Because methods used to estimate prey availability can affect null models (Cuff et al. 2024), we created two null models for each site, one using data from Malaise traps and the other using data from UV traps. We then used the function *generate_null_net* to determine whether the observed relative abundance of prey in *C. minor* fecal samples collected at that site fell within the 95% CI of expected relative abundance predicted by each of the null models (simulations = 500, datatype = quantities). Because the amount of DNA recovered in fecal samples may increase with prey size, we gave more weight to larger insects in our null models by (1) incorporating body length into estimates of expected relative abundance and (2) using RRA instead of FOO data to estimate observed relative abundance. Specifically, for (1), expected relative abundance was estimated by summing the body lengths of all individuals within each order captured in each trap type and then dividing the summed lengths of each order (a proxy for biomass) by the total for all orders (see Verkuil et al. 2022, which shows that RRAs in bird fecal DNA are highly correlated with the body lengths of insect prey). Specifically, for (2), observed relative abundance was estimated by summing the RRA for each order (calculated during diet diversity analysis) across all fecal samples. Only insect orders detected in *C. minor* diets were included in prey selection models, though some orders consumed by *C. minor* were not captured in insect traps and therefore could not be evaluated.

The function *generate_null_net* was also used to calculate the standard effect size (SES) between observed and expected prey abundance. We identified a significant difference between observed and expected relative abundances when 2 criteria were met: (1) observed abundance fell outside of the 95% CI for expected abundance; and (2) $SES > |2|$ (i.e., SES was statistically different from 0; Gotelli and McCabe 2002). A prey item was “preferred” if it was consumed significantly more than expected and “avoided” if it was consumed significantly less than expected. During the breeding season, “avoided” may also reflect items that were captured by adults and delivered to young.

RESULTS

Prey Availability

To evaluate *C. minor* diet and prey selection in the context of prey availability, we assessed aerial insect abundance, richness, and diversity at both sites. Our aerial insect traps captured 14 orders of insects at Citrus and 13 orders at Santa María (Supplemental Material S.6). Traps at both sites contained Blattodea, Coleoptera, Diptera, Ephemeroptera, Hemiptera, Hymenoptera, Lepidoptera, Mantodea, Neuroptera, Odonata, Orthoptera, Psocodea, and Trichoptera. Traps at Citrus also contained Dermaptera. Mean insect abundance was more than 6× higher in Malaise traps at Santa María compared to Citrus (345 ± 78 vs. 55 ± 13 , respectively) and over 20× higher in UV traps at Santa María than at Citrus ($11,938 \pm 3,193$ vs. 505 ± 4 , respectively). Diptera dominated Malaise traps, making up over 50% of biomass at both sites; Coleoptera dominated UV traps, making up 52% of biomass at Citrus and 74% at Santa María. Lepidoptera accounted for over 20% of Malaise and UV biomass at Citrus, but accounted for <5% of biomass at Santa María.

Diversity estimates for available prey were mostly consistent between trap types (Figure 1). Both Malaise and UV traps estimated higher richness D_α and D_γ at Santa María compared to Citrus, but Shannon and Simpson D_α and D_γ were lower at Santa María. In other words, traps captured more orders with less even biomasses at Santa María compared to Citrus. Shannon and Simpson D_β were both higher at Santa María, indicating more between-trap variation in biomass than at Citrus. UV traps had higher Sørensen and Jaccard similarities among traps at Citrus than at Santa María (Sørensen: Citrus = 0.02 ± 0.00 , Santa María = 0.01 ± 0.00 ; Jaccard: Citrus = 0.37 ± 0.01 , Santa María = 0.27 ± 0.00), but similarity indices for Malaise traps showed no differences (Sørensen: Citrus = 0.03 ± 0.00 , Santa María = 0.03 ± 0.01 ; Jaccard: Citrus = 0.50 ± 0.03 , Santa María = 0.52 ± 0.05).

Metabarcoding and Prey Classification

We obtained 9,949,645 sequences from 167 fecal samples: 71 from Citrus and 96 from Santa María (Supplemental Material S.7). We had ≥ 2 fecal samples that sequenced successfully for 8 individuals at Santa María, so we randomly selected one fecal sample from each individual for analysis. After removing duplicate samples ($n = 19$) and samples containing only *C. minor* DNA ($n = 85$), our final samples featured 13 individuals from Citrus in 2021, 15 from Citrus in 2022, 25 from Santa María in 2021, and 10 from Santa María in 2022. We removed 73 ASVs from all samples because they were present in extraction negatives.

A total of 174 ASVs matched at least one reference insect at the order level (all classified insects are listed in Supplemental Material S.8). Total mean sequencing depth was significantly higher for samples from Citrus ($100,230 \pm 11,865$ reads) than samples from Santa María ($29,514 \pm 4,256$ reads; Wilcoxon signed rank test; $p < 0.001$), even though Santa María had over twice as many insect ASVs (126 at Santa María vs. 51 at Citrus). Despite higher mean coverage, there were significantly fewer ASVs per sample at Citrus compared to Santa María for all combinations of years except between Citrus 2021 and Santa María 2022, for which $p = 0.05$; there was no difference within each site between years (pairwise Wilcoxon signed rank test; Supplemental Material S.9). While we detected similar total numbers of

orders and families across the two sites (Citrus = 17 families and 9 orders, Santa María = 19 families and 9 orders), individual fecal samples from Santa María averaged about twice as many orders and families as fecal samples from Citrus (Citrus = 1.40 ± 0.17 families and 1.32 ± 0.12 orders, Santa María = 3.09 ± 0.42 families and 2.49 ± 0.28 orders; Supplemental Material S.10).

Diet Composition and Diversity

The proportion of each order contributing to the overall diet differed based on the mode of calculation (FOO or RRA), but the relative importance of each order (i.e., which orders had the highest frequency/abundance versus the lowest frequency/abundance) differed only for Orthoptera, which had higher FOO but lower RRA than other orders at Santa María (Figure 2).

When clean and unclean samples were combined, Citrus showed significantly higher D_β than Santa María (i.e., diet contents differed more among samples within Citrus than among samples within Santa María), whereas D_α and D_γ were similar between sites (Figure 3, Supplemental Material S.11). At both sites, D_β varied between richness, Shannon, and Simpson, indicating diet unevenness between samples (i.e., prey orders were present in different quantities between samples). Within each site, D_α and D_γ were similar for richness, Shannon, and Simpson diversities, indicating diet evenness at these levels (i.e., prey orders were present in similar quantities within samples and within the population). Again, D_γ sample completeness was higher when calculated using abundance-based data (RRA; 100% completeness) than when calculated using incidence-based data (FOO; 92% completeness).

Similarity analyses indicated greater similarities between years for samples from Santa María than samples from Citrus. Stress values from NMDS analysis were ≤ 0.1 for both FOO and RRA, indicating good ordination fit (Shepard 1980). NMDS plots showed a high overlap of prey identities from samples at Santa María between years, with less overlap of samples at Citrus between years (Figures 4A, B). Citrus samples from 2021 overlapped highly with Santa María samples from both years, indicating high similarity, but Citrus samples from 2022 did not. Sørensen similarity indices for diet richness (which assessed similarity among samples within each site and year) were higher for Citrus in 2021 and 2022 than for Santa María in 2021, but large uncertainty for Santa María in 2022 inhibited detection of statistical significance for that year (Figure 4C). Sørensen similarity did not differ within sites, and Jaccard similarity did not differ between any site-year combination (Figure 4D).

Prey Selection

Prey selection by *C. minor* varied based on the trap type used to construct the null model, but both Malaise and UV models indicated similar patterns for Orthoptera, Lepidoptera, Hymenoptera, and Hemiptera (Figure 5, Supplemental Material S.12). Notably, analysis using both trap types points to a preference for Lepidoptera at Santa María by a large margin ($SES > 14$), while Lepidoptera appeared to be avoided (i.e., not consumed) at Citrus. Models constructed from both trap types also suggested that *C. minor* at Citrus preferred Hymenoptera and Hemiptera, while *C. minor* at Santa María preferred Orthoptera, Hemiptera, and Hymenoptera. Diptera and Coleoptera presented the biggest disparity in selection results between trap types: data collected using Malaise traps indicated avoidance of Diptera at both sites, while data collected using UV traps indicated a preference at both. Data for Malaise traps also suggested a preference for Coleoptera at Citrus and no selection at Santa María, while data for UV traps indicated avoidance of Coleoptera at both sites.

Nestling Provisioning

We collected five food boluses opportunistically from *C. minor* captured in mist nets at Citrus (Table 1). Hymenoptera accounted for 84.4% of all prey items found in the 5 food boluses, while Lepidoptera (the second-most abundant order) accounted for 5.6%, Coleoptera for 4.7%, Hemiptera for 2.6%, Diptera for 1.7%, and Neuroptera for $<0.1\%$.

DISCUSSION

We assessed the diets and prey communities of *C. minor* on breeding and nonbreeding sites to evaluate their prey preferences and whether these preferences changed based on prey availability and stage of the annual cycle. We hypothesized that *C. minor* at both breeding (Citrus, USA) and nonbreeding (Santa María, Argentina) sites would prefer, nutrient-rich prey such as Lepidoptera, Coleoptera, and Hymenoptera. Our results showed that *C. minor* preferred Hemiptera (true bugs) and Hymenoptera (mostly ants) in both seasons, but their preferences for Lepidoptera and Coleoptera were more nuanced. Surprisingly, *C. minor* preferred Lepidoptera on the nonbreeding grounds but not on the breeding grounds. We also hypothesized that *C. minor* would show more selectivity on the breeding grounds than on the nonbreeding grounds because of higher energy demands. Contrary to our prediction, the diversity and richness of diets did not differ within-samples or within-populations between the breeding and nonbreeding grounds, despite differences in the abundance, richness, and diversity of available prey. These results indicate that *C. minor* do not accommodate changes in energy needs and prey availability by changing their prey selectivity, as optimal foraging theory predicts. Below, we present further interpretations of our diet diversity and prey selection results, which we believe are linked to overarching differences in the ecology of breeding and nonbreeding *C. minor*, along with challenges associated with prey sampling.

Year-round Prey Preference

Chordeiles minor exhibited year-round preference for Hymenoptera and Hemiptera. This preference for Hymenoptera comports with previous studies in the northern breeding range (Brigham 1990, Brigham and Fenton 1991), but our study also provides new information on a preference for Hemiptera. All Hymenoptera consumed by *C. minor* in our study belonged to the Formicidae (ant) family, which are large-bodied and reproduce in large flying swarms at dusk and dawn (Wheeler 1910) and present *C. minor* with a means of efficiently capturing large quantities of prey. Other studies have found Hemiptera in *C. minor* stomach samples (Caccamise 1974) and food boluses (Knight et al. 2018) in the breeding grounds, but they did not evaluate prey availability. *Chordeiles minor* in our study consumed a variety of families within the Hemiptera order: Clastopteridae (spittlebugs), Cydnidae (burrowing bugs), and Pentatomidae (stink bugs) in the breeding grounds; and Cicadidae (true cicadas), Delphacidae (planthoppers), Notonectidae (backswimmers), and Rhyparochromidae (seed bugs) in the nonbreeding grounds.

Contrary to our prediction, we found that Lepidoptera were less common in fecal samples than predicted by their availability on the breeding grounds, but they were much more common than expected on the nonbreeding grounds. We suggest that *C. minor* likely captured Lepidoptera on the breeding grounds but delivered the prey to their young. Two of the 5 food boluses we recovered contained Lepidoptera (one composed of 69% and another composed of 18% Lepidoptera). Adults may selectively capture and store Lepidoptera and other nutrient-rich prey at the end of their foraging bouts to deliver to their young in the form of food boluses (adults may choose not to forage selectively for themselves, possibly explaining why Lepidoptera were not common in adult diets on the breeding grounds). This interpretation relies on the assumption that adults vary their foraging selectivity throughout the night, thus controlling which prey end up in food boluses. Other studies have also detected Lepidoptera in nestling diets of *C. minor* (Knight et al. 2018) as well as other avian insectivores (Hoenig et al. 2021, Verkuil et al. 2022, Nell et al. 2023). In contrast to our inference, we note that Knight et al. (2018) found that Coleoptera accounted for higher proportions of nestling diets than Lepidoptera in the boreal forest, which may reflect a lower relative abundance of large-bodied Lepidoptera in that environment rather than selection for Coleoptera.

The relative availability of Coleoptera varied between Malaise and UV traps, leading to

conflicting predictions about prey selection. Malaise trap data from our study suggested that Coleoptera were consumed more than expected by *C. minor* at Citrus, while UV trap data suggested that Coleoptera were consumed less than expected at both sites. However, the SES for Coleoptera was lower at Santa María than at Citrus in both models, supporting the conclusion that *C. minor* showed less preference for Coleoptera at Santa María than at Citrus. The scarcity of Coleoptera in *C. minor* diets at Santa María (found in only 4 of 35 individuals, RRA = 3%) contrasted with diet samples from other nightjars (family Caprimulgidae) at Santa María, where Coleoptera accounted for 88% of prey found in stomachs and 32% in mouths (Fariña et al. in review). Coleoptera and Lepidoptera contain some of the highest amounts of crude protein and fat relative to body size, but Coleoptera have chitinous exoskeletons, whereas Lepidoptera are easily digestible (Lease and Wolf 2010, Razeng and Watson 2015).

We were unable to determine the size of consumed prey because it requires identification to the species level (this was beyond the resolution of our DNA metabarcoding data). A previous study of *C. minor* foraging observed that individuals did not discriminate between large and small prey items, or between edible and non-edible flying targets (Brigham and Barclay 1995). Rather than discriminating between prey in flight, we infer that *C. minor* exhibit prey selection by seeking out foraging areas where preferred prey are abundant. Determining the effects of prey size on preference is an important next step for research on prey selection by aerial insectivores.

Prey Selectivity and Diet Diversity Between Seasons

We found that *C. minor* diets had similar within-individual (D_{α}) and within-population (D_{γ}) Shannon and Simpson diversities between Citrus and Santa María, even though insect communities at Citrus exhibited higher Shannon and Simpson diversities than at Santa María within-traps (D_{α}) and within-site (D_{γ}). Although Shannon and Simpson D_{α} and D_{γ} were higher for Citrus insect communities, prey community richness was higher at Santa María while diet richness was similar between sites. In other words, insect communities at Santa María contained more orders (higher richness) than communities at Citrus, but orders were present in less even abundances (lower Shannon and Simpson diversities). Although *C. minor* had access to richer prey communities at Santa María, they did not have higher diet richness; similarly, diets at Citrus did not have higher Shannon and Simpson diversities than at Santa María, even though insect communities were more diverse. We also found higher among-individual (D_{β}) diet richness and diversity at Citrus than at Santa María, even though among-trap variation in aerial insect diversity was lower at Citrus.

We found some evidence supporting our second prediction that *C. minor* preferred fewer prey orders on the breeding grounds than on the nonbreeding grounds. Prey selection models showed that individuals at Citrus preferred Hymenoptera and Hemiptera, while individuals at Santa María preferred Orthoptera, Lepidoptera, Hemiptera, and Hymenoptera. However, the sampling biases associated with Malaise and UV traps likely masked other potential patterns, particularly for Coleoptera and Diptera, for which prey selection models were contradictory depending on which trap data were used to construct the null model. For example, UV traps only attracted insects after dark and thus failed to sample prey availability during the first hour of nighthawk activity. The effectiveness of light trapping also depends on the wavelength used as insects differ in which wavelengths they are attracted to, with some insects exhibiting no phototaxis at all (low levels of phototaxis may explain the tendency of UV light traps to under-sample Diptera; Kim et al. 2019). Malaise traps, on the other hand, are not limited by time of day and use a passive sampling method that intercepts a high diversity of flying insects (Skvarla et al. 2021). However, Malaise traps are limited by their sampling height, as they only intercept insects within 2 m of the ground, and they tend to under-sample Coleoptera (which fall to the ground instead of climbing into the collection bottle; Montgomery et al. 2021).

Evaluating Diet Composition and Diversity

Analyzing diet composition using both FOO and RRA led to similar conclusions about the prominence of prey orders: overall, the orders with the highest FOO also had the highest RRA. Only Orthoptera at Santa María varied between the metrics (Orthoptera were the 4th most frequently consumed order but accounted for the 6th highest RRA). These results support other recent studies that found strong correlations between FOO and RRA in insectivore fecal samples (Wray et al. 2020, Verkuil et al. 2022). Although the quantitative data obtained from DNA metabarcoding is affected by primer biases and differences in prey digestion and sequencing depth (Alberdi et al. 2019, Jusino et al. 2019), some studies have validated the use of RRA to quantify invertebrate biomass when appropriate primers are used (Elbrecht and Leese 2015, Piñol et al. 2019, Verkuil et al. 2022). Our prey selection modeling used insect body length as a proxy for biomass in estimates of prey availability, which translates more readily to RRA than to FOO in estimates of diet composition (Cuff et al. 2024).

Whether using RRA or FOO to evaluate diet richness and diversity, it is important to standardize data based on sequencing depth (the number of DNA reads per sample) and sample completeness (the estimated percentage of true community diversity captured in sampling; McMurdie and Holmes 2014, Chao et al. 2020, Roswell et al. 2021). In our study, raw counts of order richness at the D_α level implied that *C. minor* diets at Santa María were at least twice as rich as diets at Citrus. However, once estimates were standardized, no statistical differences were observed. Similarly, aerial insect community richness and diversity estimated with Malaise and UV traps were standardized to ensure even sample completeness: sample completeness was lower at Citrus, therefore estimates were rarefied at Santa María with a method that reduced extrapolations. We also found that abundance-based methods outperformed incidence-based methods in terms of sample completeness of diet and insect community samples and, in some cases, produced smaller confidence intervals for estimates of D_V .

Conclusions

Overall, our results do not support previous research showing that aerial insectivores adjust prey selectivity to accommodate differences in energy needs or prey availability, as optimal foraging theory predicts. The discrepancy between our study and previous aerial insectivore research may be explained by differences in prey requirements between *C. minor* (a large-bodied bird for which foraging is restricted to dusk and dawn) and small birds that forage throughout the day, such as swallows and swifts. We also note that our breeding site was at a lower latitude than breeding sites in other *C. minor* studies. Low latitudes have shorter twilight periods than high latitudes and thus may provide *C. minor* with shorter foraging periods. Short foraging periods may restrict prey selectivity if the associated increase in prey search time means nighthawks cannot meet their energetic needs. Our findings suggest that *C. minor* lack diet flexibility at the latitudes studied and, thus, could be more vulnerable to changes in prey abundance and composition than other aerial insectivores, or even *C. minor* populations that breed at higher latitudes. This study was restricted to one breeding and one nonbreeding site, and the application of our interpretations to *C. minor* in other areas should be made with caution.

If migratory birds are particularly vulnerable to changes in prey communities during the breeding season, when energy needs are high, then decreases in preferred prey during breeding may decrease adult body condition, survival, productivity, and the condition and survival of young. Future studies should examine whether changes in prey communities have such effects and if these effects are more pronounced in breeding than nonbreeding birds. Additionally, we recommend that future studies examine prey selection by *C. minor* and other aerial insectivores during migration to determine their vulnerability to changing insect communities during this energy intensive time.

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Ethics statement

A federal bird banding permit was obtained from the United States Geological Survey (permit no. 22648). Permits for capturing *C. minor* and sampling insects in Florida were provided by the Florida Forest Service (LOA #2022-01-003) and Florida Fish and Wildlife Conservation Commission (permit no. LSSC-21-00020). In Argentina, *C. minor* capture and insect sampling were conducted at Reserva Natural Rincón de Santa María with permits and field site access provided by the Dirección de Parques y Reservas de la Provincia de Corrientes and the Entidad Binacional Yacyretá. Exportation and importation permits for *C. minor* fecal samples and insect PCR product were obtained from the República Argentina (permit no. IF-2021-125981324-APN-DNBI#MAD), United States Department of Agriculture (permit no. 147538), and United States Fish and Wildlife Services (permit no. MB27257C). Handling of *C. minor* followed the Louisiana State University AgCenter’s Institutional Animal Care and Use Committee (IACUC) protocol number A2021-03.

Conflict of interest statement

The authors do not declare any conflict of interest.

Author contributions

EDS, KLC, NF, OV, and SST conceived the idea, and JAC and GEZ additionally contributed to formulating the research questions and hypotheses. EDS, KLC, NF, OV, SST, JAC, GEZ, and AMS contributed to the study design and developing field and laboratory methods. EDS, NF, OV, and GEZ collected data. SST and DB contributed substantial materials and resources. EDS analyzed the data and wrote the paper, and all authors contributed to editing the paper.

Data availability

Analyses reported in this article can be reproduced using the data provided by Stein et al. (2024).

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Figure 1. Hill estimates (with 95% confidence intervals) for Shannon and Simpson diversity at the alpha (D_α) and gamma (D_γ) level were significantly higher at Citrus than at Santa Maria, but estimates at the beta (D_β) level were higher at Santa Maria. In contrast, estimates of Richness at the D_α and D_γ level were higher at Santa Maria than at Citrus, whereas estimates of richness at the D_β level were higher at Citrus. Plot background color indicates the site with the significantly higher diversity value (orange = Citrus, blue = Santa María). Note that y-axis scales differ among plots.

Figure 2. Frequencies of occurrence (left) and relative read abundances (right) for insect orders detected in *C. minor* fecal samples differed between Citrus ($n = 28$) and Santa María ($n = 35$). Frequency of occurrence was calculated for the entire population (sums are >1 because some orders were detected in multiple fecal samples). For relative read abundance, bars represent individual fecal samples; the number of orders detected in each sample increases left to right.

Figure 3. Hill numbers for *C. minor* fecal samples differ at the beta (D_β) level between Citrus (orange; $n = 28$) and Santa María (blue; $n = 35$), and between richness, Shannon, and Simpson diversities at the (D_β) level. Diversities did not differ at the alpha (D_α) or gamma (D_γ) level. Error bars show 95% confidence intervals, black brackets with asterisks denote significant differences between sites, and colored brackets with asterisks denote significant differences between diversity types. Circles with solid lines indicate that D_γ was calculated using abundance-based data (RRA), whereas triangles with dashed lines indicate that D_γ was calculated using incidence-based data (FOO).

Figure 4. Diet similarities across sites and years. Top: Diet ordination based on non-metric multidimensional scaling, calculated using relative read abundance (a) and frequency of occurrence (b). Dots are unique samples (some very similar dots overlap), and ellipses provide 95% confidence levels for ordinations. Axes provide scales for rank-ordered similarity among points. Bottom: Sørensen (c) and Jaccard (d) similarity indices for diet richness among samples within each site and year. Dots represent means of 50 bootstrap replications, and error bars represent 95% confidence intervals. Red = Citrus 2021 ($n = 13$), orange = Citrus 2022 ($n = 15$), light blue = Santa María 2021 ($n = 25$), dark blue = Santa María 2022 ($n = 10$).

Figure 5. Standard effect size (SES) of the observed relative abundance of insects detected in *C. minor* fecal samples compared to the expected relative abundance based on insect samples collected using Malaise traps (left) and UV traps (right). Red and blue points indicate an observed value falling above (red) or below (blue) the 95% confidence interval (CI) of the expectation under the null model, while white points fall within the 95% CI. Points with $SES > |2|$ (dashed lines) have an effect size significantly different from 0.

Table 1. Number and percentage (%) of individuals from each insect order found in adult *C. minor* food boluses collected during the breeding season at Citrus.

	9 July 2021	13 June 2022	26 July 2021	1 July 2022	5 July 2022
Insect order	(Female)	(Female)	(Female)	(Female)	(Male)
Coleoptera	3 (18.8%)	7 (60%)	–	1 (9.09%)	–
Diptera	–	1 (10%)	–	3 (27.3%)	–
Hemiptera	1 (6.25%)	1 (10%)	–	4 (36.4%)	–
Hymenoptera	–	1 (20%)	37 (100%)	1 (9.09%)	155 (100%)
Lepidoptera	11 (68.8%)	–	–	2 (18.2%)	–
Neuroptera	1 (6.25%)	–	–	–	–

Figure 1

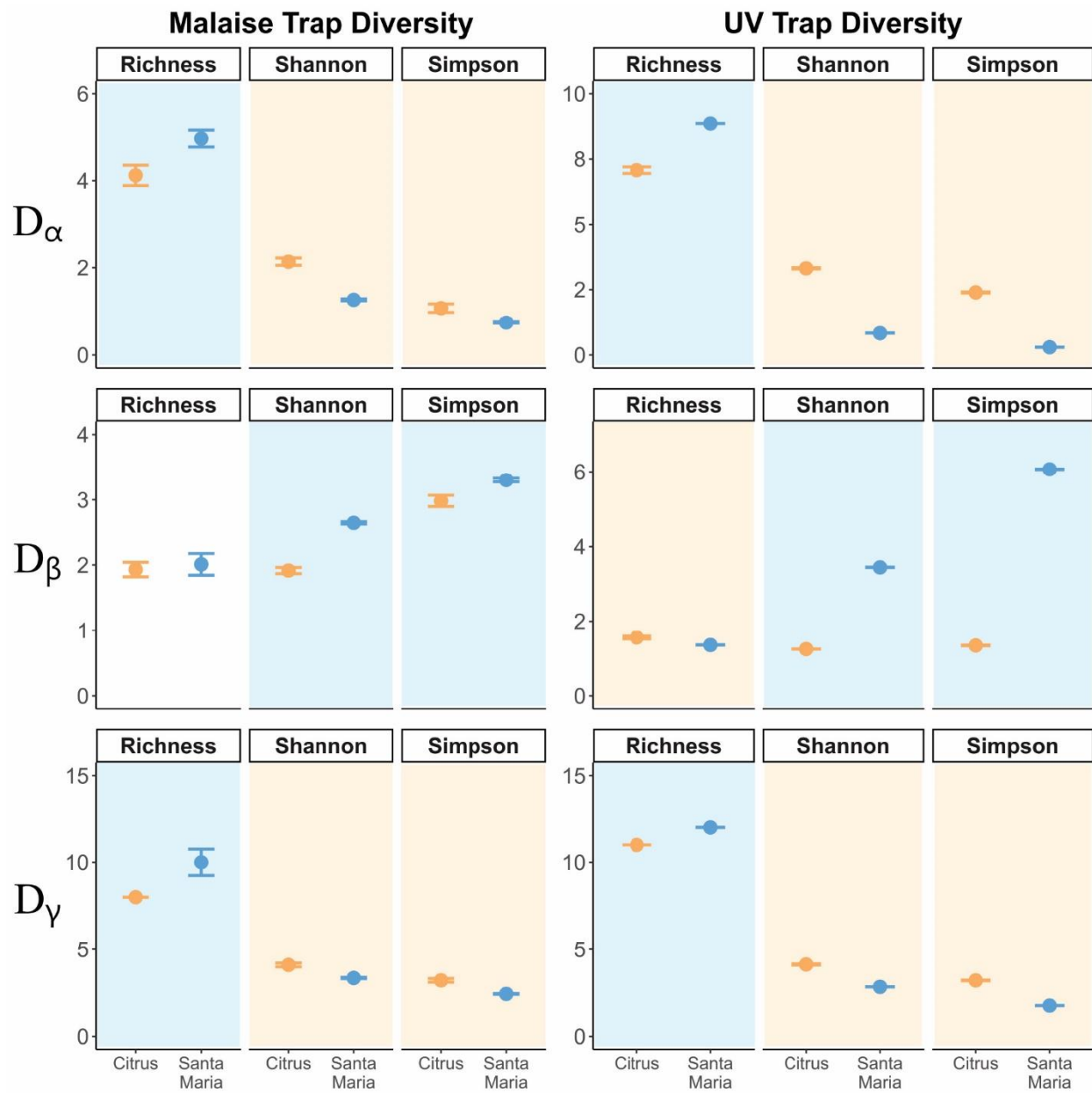


Figure 2

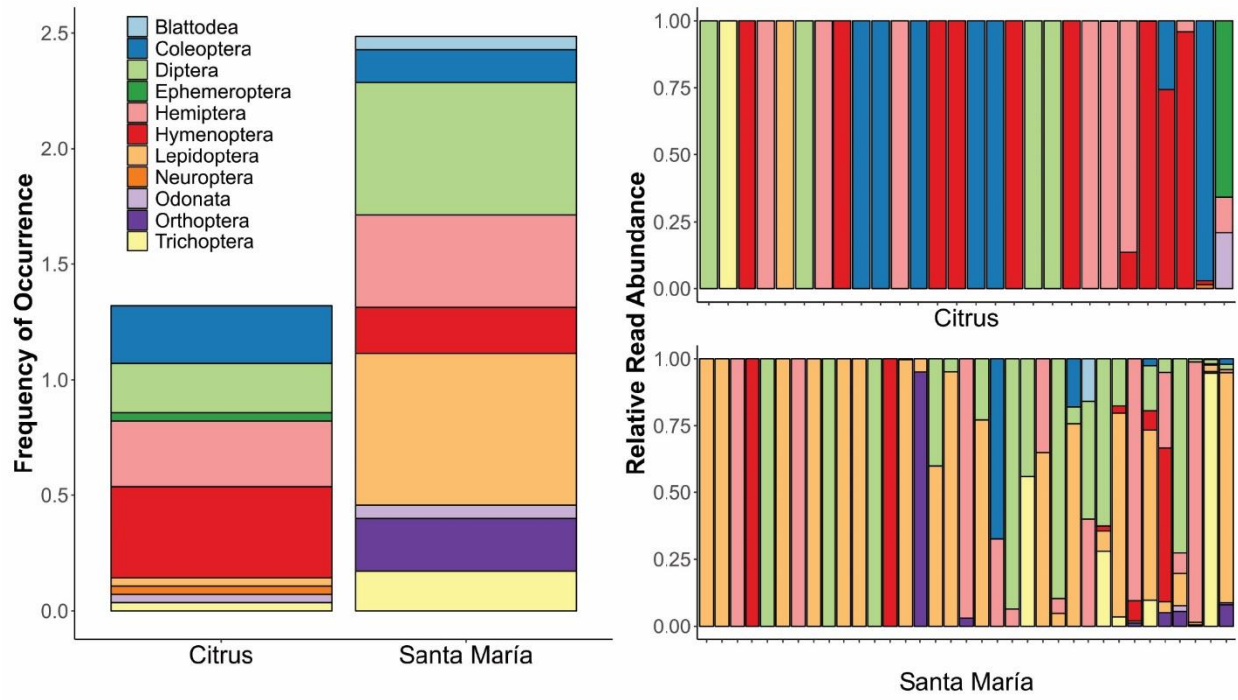


Figure 3

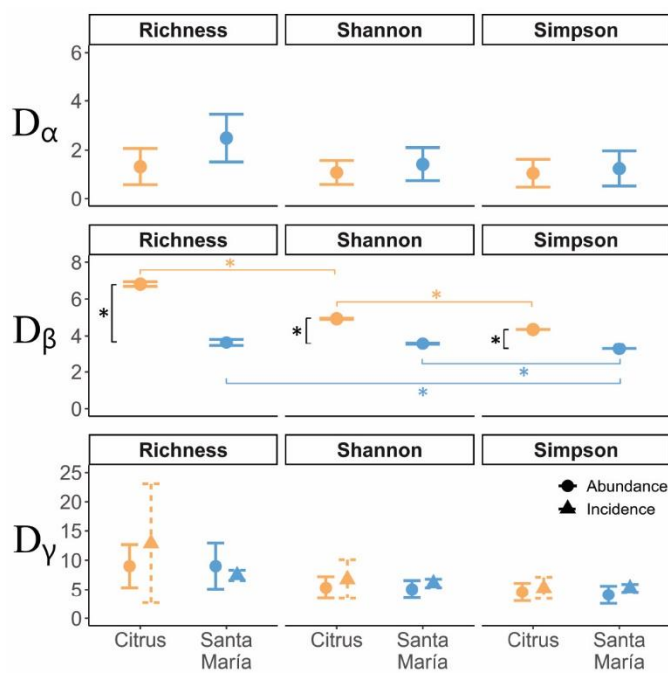


Figure 4

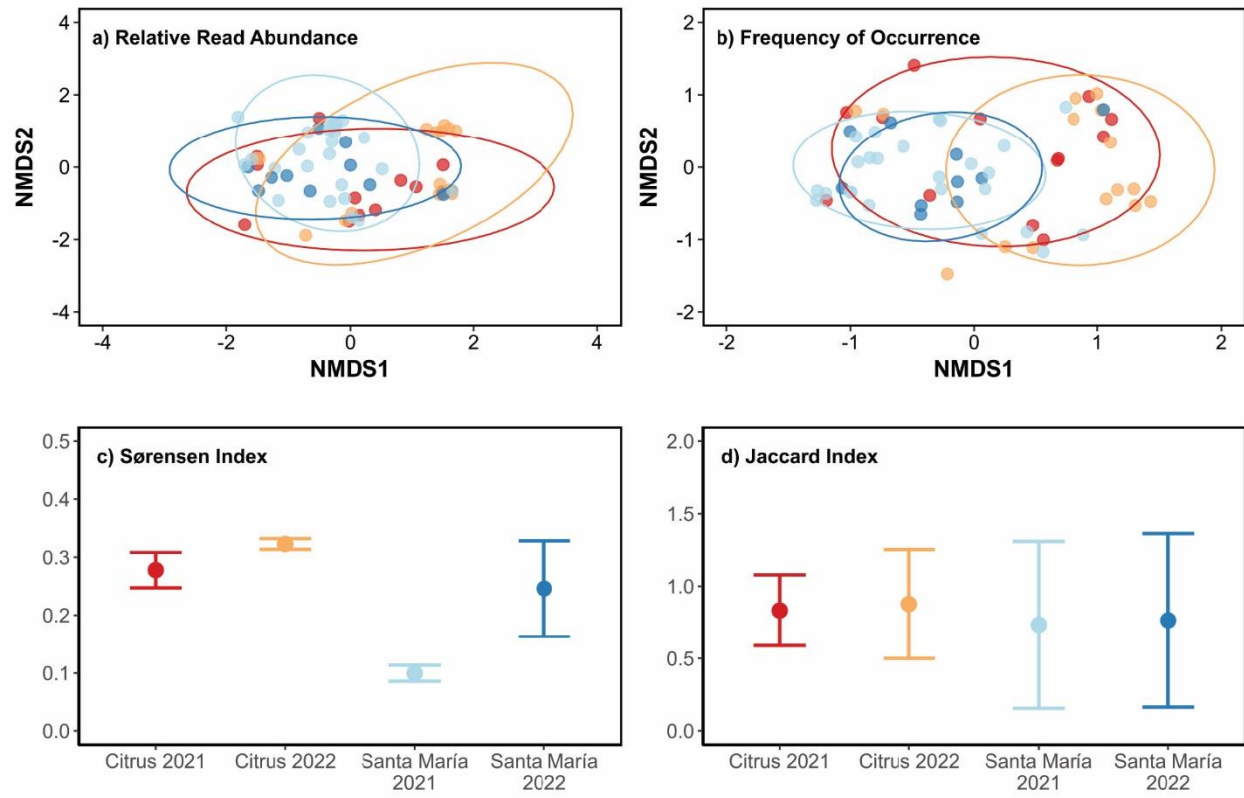


Figure 5

