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Predator preferences shape the diets of arthropodivorous bats more than quantitative local prey abundance

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Abstract

Although most predators are generalists, the majority of studies on the association between prey availability and prey consumption have focused on specialist predators. To investigate the role of highly generalist predators in a complex food web, we measured the relationships between prey consumption and prey availability in two common arthropodivorous bats. Specifically, we used high-throughput amplicon sequencing coupled with a known mock community to characterize seasonal changes in little brown and big brown bat diets. We then linked spatiotemporal variation in prey consumption with quantitative prey availability estimated from intensive prey community sampling. We found that although quantitative prey availability fluctuated substantially over space and time, the most commonly consumed prey items were consistently detected in bat diets independently of their respective abundance. Positive relationships between prey abundance and probability of consumption were found only among prey groups that were less frequently detected in bat diets. While the probability of prey consumption was largely unrelated to abundance, the community structure of prey detected in bat diets was influenced by the local or regional abundance of prey. Observed patterns suggest that while little brown and big brown bats maintain preferences for particular prey independently of quantitative prey availability, total dietary composition may reflect some degree of opportunistic foraging. Overall, our findings suggest that generalist predators can display strong prey preferences that persist despite quantitative changes in prey availability.

KEYWORDS
amplicon sequencing, Chiroptera, diet analysis, DNA metabarcoding, food webs, mock community

1 | INTRODUCTION

Though capable of consuming many types of prey resources, generalist predators are often selective. Predator preference, that is, any deviation from a random sampling of available prey, is a particularly useful measure for describing which prey resources are sought out or avoided by a selective predator (Chesson, 1978; Manly et al., 1972). Under the assumption that foraging is optimal, predator preferences for certain prey are expected to maximize net energy gains, and consequently, consumption of nonpreferred prey should be expected only when preferred prey are absent or low in abundance (Pulliam, 1974; Schoener, 1971). However, empirical studies often demonstrate patterns that deviate from optimal foraging, particularly for predators that consume mobile prey (Sih & Christensen, 2001).

Additionally, despite the importance of understanding how predator preferences influence their role in food webs, many generalist predators lack detailed descriptions of the prey that they consume, and even fewer have corresponding information on their responses to fluctuating resource availability (Pringle, 2020; Thompson et al., 2012).

For generalist predators, prey preferences can influence functional responses (sensu Holling, 1959a,1959b), although the relationship between prey consumption and prey availability may not necessarily resemble classic response types (Murdoch & Oaten, 1975; Oksanen et al., 2001). For example, generalist predators foraging in multiprey systems have been shown to consume prey irrespective of changing availability or even reduce the consumption of increasing prey as a result of preferences related to nutritional demands, energy requirements or ease of prey capture (Baudrot et al., 2016; Chesson, 1984; Dale et al., 1994). Therefore, the relationship between prey consumption and quantitative availability in generalist predators can be highly context-dependent (Novak et al., 2017; Preston et al., 2018; Symondson et al., 2002). The occurrence of individual specialization, even among the most archetypal generalist predators, also suggests that prey preferences are not necessarily static over time or between conspecifics (Bolnick et al., 2002; Panzacchi et al., 2008; Sacks & Neale, 2002; Woo et al., 2008). Recent studies have provided additional evidence that generalist predators may display consistent selectivity despite low preferred prey availability or abundant alternative prey (Krey et al., 2017; Ritger et al., 2020; Roubinet et al., 2018; Whitney et al., 2018). Overall, the growing body of empirical studies on generalist predators suggests that prey preferences can influence foraging patterns, perhaps to a greater extent than previously thought.

The manner in which prey preferences shape foraging behaviours has important implications for the extent to which generalist predators exert top-down effects on prey communities and ecosystems. Previous studies that have characterized generalist predators in terms of biocontrol potential, responses to habitat loss and stabilization of food webs have yielded conflicting results. In some studies, generalist predators have been shown to decrease pest populations, persist in fragmented habitat or increase food web stability, while others have demonstrated the opposite (Prugh, 2005; Ryall & Fahrig, 2006; Snyder & Wise, 1999; Symondson et al., 2002). As generalist predators thus have unpredictable effects on their food webs, which can also change both spatially and temporally, continuing to develop and test predictions regarding their responses to changing prey communities remains essential for both theoretical and applied ecology.

Among generalist predators, arthropodivorous vertebrates are unique as they usually consume many more prey types than obligate carnivores while maintaining much higher energy requirements than predaceous arthropods. Moreover, exclusion studies have demonstrated that arthropodivorous vertebrates can have a range of both direct and indirect effects on their respective food webs (Mooney et al., 2010). The mechanisms governing these responses are less well understood, partly due to the difficulties in defining the full suite of prey resources that are consumed by these predators. Among terrestrial arthropodivorous vertebrates, most studies relating prey preferences and prey abundance have used morphological methods that characterize predator diet composition by visual inspection of stomach contents or faecal material (Ralph et al., 1985; Whitaker et al., 2009). However, these methods are limited in taxonomic resolution and prey remains are often damaged or degraded, particularly for soft-bodied arthropods.

Newer techniques such as high-throughput amplicon sequencing (HTAS) can characterize animal diets at a much finer taxonomic resolution than other methods, providing a more comprehensive way to study the entire suite of prey resources consumed by generalist predators (Jusino et al., 2019; Kaartinen et al., 2010; Zeale et al., 2011). Since the advent of these methods, many studies have described the diets of a wide range of taxa (Alberdi et al., 2019, 2020; Piñol et al., 2014; Pompanon et al., 2012; Wray et al., 2018). These types of studies collectively represent an important first step in describing animal diets, especially for those that use a broader diversity of prey resources than other methods that are capable of detecting. For arthropodivorous vertebrates in particular, studies using molecular methods have generated a wealth of detailed data on dietary composition and dietary breadth, and relating these data to underlying prey abundance can reveal key insights into predator foraging (e.g. Arrizabalaga-Escudero et al., 2019; Vesterinen et al., 2016). As many arthropodivorous vertebrates, particularly birds and bats, are currently threatened due to habitat loss, arthropod declines and disease, among other factors (Nebel et al., 2010; O'Shea et al., 2016; Rioux Paquette et al., 2014; Spiller & Dettmers, 2019), fully characterizing their resource requirements also represents a timely endeavour.

Bats have been the subject of benchmark studies on the use of molecular methods for characterizing the diets of generalist predators (Clare et al., 2011; Clare, Symondson, & Broders, 2014; Clare, Symondson, & Fenton, 2014; Razgour et al., 2011), largely due to interest in the potential ecosystem services they provide in the form of agricultural pest reduction (Boyles et al., 2011; Kunz et al., 2011). The diets of two common North American bats—the little brown (*Myotis lucifugus*, Leconte 1831) and big brown bat (*Eptesicus fuscus*, Palisot de Beauvois 1796)—have been especially well described using both molecular and morphological methods, and as such, these species serve as a useful model for studying generalist arthropodivore foraging. The preferred prey of little brown and big brown bats, respectively, are frequently reported as small aquatic insects and beetles (Agosta, 2002; Fenton, 1980; Kurta & Baker, 1990; Moosman et al., 2012), though little brown bats have been observed switching to opportunistic foraging in response to changing prey abundances (Anthony & Kunz, 1977; Belwood & Fenton, 1976; Burles et al., 2008). Several molecular studies on other bat species have related prey consumption to quantitative prey abundance (Baroja et al., 2019; Krauel, Brown, et al., 2018; Krauel, Ratcliffe, et al., 2018; Weier et al., 2019), but often included select prey groups of interest rather than intensively sampled prey communities (e.g. Vesterinen et al., 2016). For

little brown and big brown bats, however, dietary composition data from molecular methods have not yet been connected with underlying prey abundance information.

In this study, we characterized how prey consumption by two generalist arthropod predators, the little brown and big brown bat, changes in response to quantitative spatiotemporal variation in prey resources. We hypothesized that highly mobile generalist predators would display preferences for certain prey and that the probability of consuming most prey groups would not increase as a direct function of increasing abundance. We predicted that for both little brown and big brown bats, the probability of detecting prey in guano samples would not increase as a direct function of increasing quantitative prey availability as measured by arthropod traps. We tested these predictions by comparing HTAS dietary data from guano samples with quantitative arthropod abundance estimated from black-light traps that captured arthropods during the same time periods at locations near bat maternity roosts. From these analyses, we inferred which prey were preferred with respect to their relative abundance. As an exploratory analysis, we also assessed how dietary diversity related to underlying prey diversity and how regional or local arthropod abundance influenced dietary composition. Overall, both bat species appeared to exhibit strong preferences for certain prey groups, and the quantitative availability of most prey groups was unrelated to the probability of detection in bat guano samples.

2 | MATERIALS AND METHODS

2.1 | Bat guano collection

Study sites were selected at 6 little brown and 4 big brown bat maternity roosts in southern Wisconsin (Figure S1). These sites were located at state and county parks and privately owned land, and were selected using the following criteria: (a) sites included a known maternity roost with bats of a visually confirmed species that consistently returned for several consecutive years; (b) sites were accessible and sampling requests were approved by land owners or managers; (c) habitat composition represented a gradient of agricultural and forest land cover; and (d) bat roosts were included in pre- and postvolancy bat count efforts conducted by volunteers as part of other state-wide monitoring efforts. Based on emergence counts from previous surveys in 2015, big brown bat colonies ranged from approximately 28–287 individuals, while little brown bat colonies ranged from approximately 89–446 individuals. Landscape conditions at these study sites included a range of habitat types, which were assessed using the USDA National Agricultural Statistics Service Cropland Data Layer (https://nassgeodata.gmu.edu/CropScape/). Within a 3-km radius, the average landscape composition was 33% agricultural (ranging from 7.9 to 58%), 30% forest (ranging from 3.0 to 63%), 20% grass or pasture (ranging from 4.4 to 42%) and 11% wetland or open water (ranging from 0.15 to 40%). All sites were located near bodies of water (including small ponds, lakes and streams of varying sizes), which are common throughout the study area.

We chose to use noninvasively collected bat guano samples collected beneath roosts to allow simultaneous sample collection at multiple sites and to avoid disturbing bats during the breeding season. Bat guano was collected weekly, with fresh pellets assumed to represent the weekly prey consumption of a bat colony at each given roost. Bat species were confirmed visually each week, and pellet identity was also confirmed based on size. Clean plastic sheets were placed under each roost for 1 week, with guano samples collected from late May to late August in 2015 (Julian weeks 24–35) and mid-May to early September in 2016 (Julian weeks 23–37). Samples were initially stored at −20°C and then transferred to −80°C for long-term storage. All sample collection methods were carried out in accordance with Wisconsin Department of Natural Resources guidelines, and experimental protocols were approved by the Wisconsin Natural History Inventory (NHI) Program and the University of Wisconsin-Madison.

2.2 | DNA extraction, PCR and library preparation

A subsample of 80 mg (~8 pellets) was selected from each guano sample for genetic analyses. DNA was extracted from each guano subsample using a QIAGEN DNA Stool Mini Kit (QIAGEN), following the manufacturer's protocols except for the following changes: 10 ml ASL lysis buffer was added to 80 mg guano, vortexed for 2 min, lysed for an additional 5–10 min and centrifuged at 9391 *g* for 5 min before taking 1.8 ml of the lysate. Additionally, 40 µl of proteinase K was used per extraction instead of 10 µl. Following DNA extraction, a 180-bp cytochrome oxidase C subunit 1 (COI) amplicon, the DNA barcode region generally used for arthropods, was amplified using the ANML primer pair (FWD: GGTCAACAAATCATAAAGATATTGG; REV: GGWACTAATCAATTTCCAAATCC) according to Jusino et al. (2019). This primer pair was previously validated and was shown to have less taxonomic bias than any other primers currently available for HTAS of arthropodivore diets (Jusino et al., 2019). Primers were modified for HTAS by adding a unique barcode sequence and an Ion Torrent Xpress A adapter sequence on each forward primer, and an Ion Torrent Express trP1 adapter on the reverse primer. To overcome issues with amplification, sample DNA templates were tested at full concentration, then tested at serial dilutions of 1:10, 1:20 and 1:40. For each sequencing library, a single-copy mock community of 34 known arthropod constituents was separately amplified under the same PCR conditions as a positive control (Jusino et al., 2019). Negative controls for each DNA extraction batch and for each PCR master mix were also tested and visualized on a 2% agarose gel. These negative controls did not demonstrate visible bands.

For library preparation, all PCR products were individually purified using a Zymo Select-A-Size DNA Clean & Concentrator Kit (Zymo Research). The concentration of each purified PCR product

was quantified using a Qubit dsDNA High Sensitivity Assay with a Qubit Fluorometer (Invitrogen). Purified PCR products were then combined in equimolar amounts for a sequencing library with a final concentration of 2000 pM. Libraries were sequenced with a 400 bp Hi-Q Kit on an Ion Torrent Personal Genome Machine next-generation sequencing platform (PGM; Thermo Fisher) with a 318 chip according to manufacturer's recommendations. A total of three libraries were sequenced consisting of approximately 72 unique barcoded samples each. Samples from different sites were processed in a randomized order, and samples from both bat species were also extracted, amplified and sequenced together in order to reduce potential batch effects (Alberdi et al., 2019).

2.3 | Bioinformatics

Data from all three sequenced libraries were combined and processed cumulatively using AMPtk v1.4.0 (Palmer et al., 2018). Raw sequence data were demultiplexed using the unique barcode index sequences, and forward and reverse primers were trimmed from the 180-bp amplicon target. Measures for quality control included removal of reads shorter than 170 bp or longer than 180 bp and removal of samples with fewer than 4,000 reads. The DADA2 clustering algorithm (Callahan et al., 2016) was then used for denoising and quality filtering with expected error trimming. The resulting amplicon sequence variants (ASVs) were clustered using the UCLUST algorithm employed in VSEARCH at 97% similarity to generate operational taxonomic units (OTUs) approximating species-level taxonomy (Jusino et al., 2019). Demultiplexed sequences were mapped back onto these OTU representative sequences, and the 34-member single-copy arthropod mock community was used to account for barcode switching (also referred to as index bleed). OTUs were then assigned taxonomy using the built-in curated COI database in AMPtk, and all OTUs that were not designated as arthropods or identified beyond Arthropoda were manually removed (n = 153 OTUs).

2.4 | Data preparation

Richness in bat diet was calculated as the total number of unique arthropod groups at different taxonomic levels (OTUs, species, genera and families). To assess sufficiency of sampling intensity, accumulation curves for total family-level richness with extrapolations were created for both bat species using the R package 'iNEXT' (Hsieh et al., 2016). Following taxon assignments and clustering, OTU tables were aggregated at the family, genus and species levels. For comparison with black-light trap data, OTU tables were also aggregated into the same focal groups (Table 1). Weighted per cent of occurrence (wPO, a presence/absence-based metric where read counts are converted to binary responses) and relative read abundance (RRA, a read-based metric that incorporates the total number of DNA sequence counts) were calculated following Deagle et al., 2019.

TABLE 1 Focal families for black-light and guano samples

Order	Subgroup	Common name
Araneae	None	Spiders
Coleoptera	Carabidae	Ground beetles
Coleoptera	Coccinellidae: Harmonia	Lady beetles
Coleoptera	Elateridae	Click beetles
Coleoptera	Hydrophilidae	Water scavenger beetles
Coleoptera	Scarabaeidae	Scarab beetles
Coleoptera	Scarabaeidae: Phyllophaga	June beetles
Coleoptera	Silphidae	Carrion beetles
Coleoptera	Staphylinidae	Rove beetles
Diptera	Culicidae & Chironomidae	Mosquitoes & midges
Diptera	Muscidae	House flies
Diptera	Sarcophagidae	Flesh flies
Diptera	Syrphidae	Hover flies
Diptera	Tachinidae	Tachinid flies
Hemiptera	Cicadellidae	Leafhoppers
Hemiptera	Corixidae	Water boatmen
Hemiptera	Miridae	Plant bugs
Hymenoptera	Braconidae	Braconid wasps
Hymenoptera	Formicidae	Ants
Hymenoptera	Ichneumonidae	Ichneumonid wasps
Lepidoptera	Arctiidae	Tiger moths
Lepidoptera	Geometridae	Geometer moths
Lepidoptera	Lasiocampidae	Lappet moths
Lepidoptera	Micromoth	Micromoths
Lepidoptera	Noctuidae	Owlet moths
Lepidoptera	Sphingidae	Sphinx moths
Neuroptera	Chrysopidae & Hemerobiidae	Lacewings
Opiliones	None	Harvestmen
Orthoptera	None	Grasshoppers, crickets, katydids
Parasitiformes	None	Ticks & mites
Plecoptera	None	Stoneflies
Trichoptera	None	Caddisflies

2.5 | Arthropod trapping and enumeration

At each of the 10 aforementioned bat roost sites, arthropod communities were sampled weekly to quantify the available prey at each site during the same time interval when guano samples were collected. Black-light traps were used to collect night-flying arthropods that are presumed to form the majority of the prey consumed by arthropodivorous bats that usually forage by aerial

hawking, such as the two species in this study. Black-light traps were placed in open areas away from main roads or paths at a distance of 50–100 m from each of the bat maternity roost sites. Any vegetation surrounding the black-light trap was cleared weekly to prevent obscuration of the trap. The immediate areas of black-light trap placement included a range of vegetation such as cropland (e.g. alfalfa and corn), grassland (e.g. idle grazing land or restored prairie) or near forest edges. Traps were not placed in forest interiors to avoid blocking or reducing the visibility of the lights. Black-light traps consisted of a 3.5 gallon polypropylene bucket with a 30-cm aluminium funnel and mesh collecting bag (BioQuip Universal Light Trap, catalog number 2851). A 12-watt U-shaped bulb was affixed between three clear acrylic vanes on top of the funnel, and an aluminium lid was secured with bungee cords. An 18.6% dichlorvos (2,2-dichlorovinyl dimethyl phosphate) insecticide strip (Hot Shot No-Pest Strip2) was affixed inside the bucket. Pest strips were changed every 4 weeks to ensure equally high potency over time. Black-light traps were powered by a 12 V sealed lead acid battery, which was recharged by an attached 45-watt solar panel. Traps were turned on automatically from 20:00 through 5:00 for a consecutive 3-night period for each sampling week. Samples were collected, and traps were reset weekly from mid-May to late August in 2015 and mid-May to early September in 2016.

Arthropod samples were identified by microscope in the following manner: large or noticeably unique specimens were first selected from the overall sample for identification, and then, the remaining sample was scanned for any specimens that were not homogenous through the entire sample, which were then also selected for separate identification. For samples containing very large numbers of individuals, the homogenous remainder was divided into a subsample for identification, then extrapolated based on the portion taken to obtain an estimate of the whole sample quantity. The selected specimens and subsamples were identified to order; and within orders, all specimens were identified to the 32 most commonly detected groups (representing 95% of all captured arthropods), with remaining rare families identified as 'other Order', for example 'other Coleoptera' (Table 1). Samples that were damaged or degraded were identified to the lowest taxonomic level possible. Enumeration of identified arthropods was conducted by visual counting with the use of a multiple unit tally counter. Arthropod identifications and DNA library preparations were performed in separate laboratories in order to avoid cross-contamination.

2.6 | Statistical analyses

Both read-based and presence-based taxonomy tables were used for describing dietary composition for each bat species. For statistical analyses comparing dietary differences between little brown and big brown bats, the OTU table (Table S2) was converted to a presence/absence matrix. Interspecific dietary composition was initially

TABLE 2A Top OTUs detected in guano samples, little brown bat (*Myotis lucifugus*)

OTU ID	Order	Family	Genus	Species	Incidence	Mean wPO	Mean RRA
OTU12	Ephemeroptera	Caenidae	*Caenis*	*Caenis amica*	42	0.014	0.059
OTU13	Diptera	Chironomidae	*Procladius*		30	0.01	0.035
OTU20	Hemiptera	Corixidae	*Trichocorixa*	*Trichocorixa borealis*	27	0.009	0.021
OTU41	Diptera	Psychodidae	*Psychoda*	*Psychoda alternata*	25	0.008	0.006
OTU7	Diptera	Chironomidae	*Coelotanypus*		22	0.008	0.027
OTU143	Diptera	Chironomidae			20	0.007	0.006
OTU15	Lepidoptera	Depressariidae	*Agonopterix*	*Agonopterix robiniella*	20	0.008	0.05
OTU158	Lepidoptera	Tortricidae	*Acleris*	*Acleris semipurpurana*	19	0.005	0.002
OTU2	Diptera	Chironomidae	*Tanypus*		19	0.011	0.046
OTU145	Ephemeroptera	Caenidae	*Caenis*	*Caenis amica*	18	0.006	0.005
OTU55	Diptera	Chironomidae	*Cryptochironomus*		18	0.005	0.006
OTU22	Diptera	Chironomidae	*Chironomus*		17	0.006	0.015
OTU30	Hymenoptera				17	0.007	0.019
OTU1460	Hymenoptera	Apidae	*Apis*	*Apis mellifera*	16	0.005	0.001
OTU503	Lepidoptera	Tortricidae	*Proteoteras*	*Proteoteras crescentana*	16	0.003	<0.001
OTU120	Hymenoptera				15	0.004	0.004
OTU150	Diptera	Limoniidae	*Geranomyia*		15	0.004	0.002
OTU385	Diptera	Chironomidae	*Glyptotendipes*	*Glyptotendipes meridionalis*	15	0.004	<0.001
OTU476	Diptera				15	0.004	<0.001
OTU654	Hymenoptera	Apidae	*Apis*	*Apis mellifera*	15	0.005	<0.001

TABLE 2B Top OTUs detected in guano samples, big brown bat (*Eptesicus fuscus*)

OTU ID	Order	Family	Genus	Species	Incidence	Mean wPO	Mean RRA
OTU9	Coleoptera	Elateridae	*Melanotus*	*Melanotus similis*	25	0.017	0.064
OTU1	Trichoptera	Hydropsychidae	*Potamyia*	*Potamyia flava*	22	0.024	0.194
OTU21	Coleoptera	Elateridae	*Hemicrepidius*	*Hemicrepidius memnonius*	19	0.011	0.025
OTU193	Coleoptera	Carabidae	*Agonum*	*Agonum placidum*	16	0.008	0.002
OTU1314	Coleoptera	Elateridae	*Hemicrepidius*	*Hemicrepidius memnonius*	14	0.007	0.001
OTU123	Coleoptera	Carabidae	*Notiobia*	*Notiobia terminata*	13	0.006	0.002
OTU148	Coleoptera	Carabidae	*Harpalus*	*Harpalus pensylvanicus*	13	0.006	0.005
OTU282	Coleoptera	Carabidae	*Harpalus*		13	0.007	0.001
OTU204	Coleoptera				12	0.006	0.001
OTU3	Diptera	Limoniidae			12	0.008	0.051
OTU429	Hymenoptera	Ichneumonidae	*Enicospilus*		12	0.006	<0.001
OTU629	Coleoptera	Cantharidae	*Rhagonycha*	*Rhagonycha lignosa*	12	0.006	<0.001
OTU1445	Diptera				11	0.005	<0.001
OTU255	Diptera	Tipulidae	*Nephrotoma*	*Nephrotoma ferruginea*	11	0.009	0.001
OTU34	Diptera	Sciaridae			11	0.007	0.007
OTU38	Megaloptera	Corydalidae	*Chauliodes*	*Chauliodes pectinicornis*	11	0.005	0.046
OTU993	Coleoptera	Elateridae	*Hemicrepidius*	*Hemicrepidius memnonius*	11	0.005	<0.001
OTU30	Hymenoptera				10	0.006	0.011
OTU20	Hemiptera	Corixidae	*Trichocorixa*	*Trichocorixa borealis*	9	0.007	0.016
OTU23	Coleoptera	Pyrochroidae	*Dendroides*	*Dendroides canadensis*	9	0.007	0.015

assessed using a two-way ANOVA including bat species (with little brown bats as the reference group), prey order, and the interaction between bat species and prey order as independent variables, and the OTU richness within a family as the dependent variable. To test for overall trends in prey communities, differences in prey group abundances were analysed using Welch's *t* tests (with year-to-year comparisons constrained to Julian weeks 24–35 to account for differences in sampling season length).

We used binary logistic regressions, coded as generalized linear models (GLMs) with a logit link function, to test for potential relationships between the abundance of arthropod taxa and their probability of detection in bat diets, conducting separate analyses for the two bat species. The putative presence/absence of an arthropod taxa group was treated as the dependent variables, while the same arthropod taxa group and its respective abundance in given black-light trap were treated as the independent variables. Samples with arthropod abundance in excess of 10,000 individuals from the same arthropod group were excluded, and the remaining arthropod abundance data were normalized using a log base 10 transformation. A global model was structured such that the arthropod taxa group represented the main effect, while the respective abundance represented within each arthropod taxonomic group represented the interaction effect, *that* is:

$$Y = a + b_1X_1 + b_2X_1X_2 + e$$

where Y represents the binary presence/absence of an arthropod taxa group in a sample, X_1 is the group, and X_2 is the corresponding abundance of that particular group. Collection site, Julian week, and year were also included in the global model, and were then sequentially removed from the model when terms were not statistically significant as determined by the Wald tests. Araneae was chosen as the reference variable for arthropod groups because this group was neither common nor rare in black-light traps or in the diets of both bat species in this study, and the reported positive and negative effects are relative to this group. Little brown and big brown bats are typically thought to forage primarily by aerial hawking, although both display some flexibility in foraging strategies and can glean nonflying prey such as Araneae (Kurta & Baker, 1990; Ratcliffe and Dawson, 2003). For interaction terms, the reported slope β_{int} represents the slope of the interaction effect only, and *OR* represents the odds ratio of the main effect combined with the interaction.

To assess the relationships between dietary diversity and underlying prey abundance, we compared Hill numbers in bat guano samples with Hill numbers in corresponding black-light trap samples. For both bat guano and black-light trap samples, Hill numbers were calculated based on relative abundance following Alberdi & Gilbert, 2019 and were assessed at the orders of diversity for $q = 0$ (richness), $q = 1$ (Shannon diversity) and $q = 2$ (Simpson diversity). To compare the similarities between bat diet composition, we also calculated family-level Sørenson turnover using the R package 'vegetarian' (Charney & Record, 2015). Turnover was calculated for both read-based (RRA) and presence-based (wPO) metrics for all samples

TABLE 3 Ranking of top families detected in guano samples by different measures.

	Richness			Incidence			Mean wPO			Mean RRA		
	Order	Family	Value	Order	Family	Value	Order	Family	Value	Order	Family	Value
MYLU	Diptera	Chironomidae	156	Diptera	Chironomidae	96	Diptera	Chironomidae	0.065	Diptera	Chironomidae	0.218
	Diptera		86	Diptera		71	Hymenoptera		0.045	Hymenoptera		0.071
	Hymenoptera		54	Hymenoptera		63	Diptera		0.038	Ephemeroptera	Caenidae	0.067
	Lepidoptera	Tortricidae	50	Lepidoptera	Tortricidae	62	Lepidoptera	Tortricidae	0.033	Lepidoptera	Depressariidae	0.059
	Lepidoptera		44	Diptera	Limoniidae	59	Lepidoptera		0.03	Diptera	Limoniidae	0.043
	Coleoptera		40	Lepidoptera		55	Diptera	Limoniidae	0.03	Lepidoptera	Tineidae	0.038
	Diptera	Limoniidae	34	Ephemeroptera	Caenidae	47	Ephemeroptera	Caenidae	0.027	Trichoptera	Hydropsychidae	0.033
	Hemiptera	Miridae	25	Coleoptera		46	Coleoptera	Elateridae	0.026	Diptera		0.033
	Lepidoptera	Gelechiidae	25	Coleoptera	Elateridae	43	Coleoptera		0.026	Diptera	Psychodidae	0.032
	Diptera	Ceratopogonidae	22	Lepidoptera	Gelechiidae	43	Diptera	Psychodidae	0.023	Hemiptera	Corixidae	0.03
EPFU	Coleoptera		54	Coleoptera		39	Coleoptera		0.045	Trichoptera	Hydropsychidae	0.195
	Lepidoptera		43	Coleoptera	Carabidae	39	Coleoptera	Carabidae	0.041	Coleoptera	Elateridae	0.119
	Diptera		38	Diptera	Limoniidae	37	Coleoptera	Elateridae	0.04	Diptera	Limoniidae	0.107
	Diptera	Chironomidae	34	Diptera		36	Trichoptera	Hydropsychidae	0.04	Coleoptera	Scarabaeidae	0.065
	Hymenoptera		34	Coleoptera	Elateridae	36	Diptera		0.039	Megaloptera	Corydalidae	0.061
	Coleoptera	Carabidae	28	Lepidoptera		32	Diptera	Limoniidae	0.039	Ephemeroptera	Heptageniidae	0.051
	Diptera	Limoniidae	27	Trichoptera	Hydropsychidae	29	Lepidoptera		0.037	Hymenoptera		0.046
	Lepidoptera	Tortricidae	25	Hymenoptera		28	Diptera	Chironomidae	0.031	Diptera	Sepsidae	0.031
	Coleoptera	Elateridae	22	Diptera	Chironomidae	25	Hymenoptera		0.03	Diptera	Chironomidae	0.03
	Coleoptera	Cerambycidae	19	Coleoptera	Scarabaeidae	25	Coleoptera	Scarabaeidae	0.027	Lepidoptera	Tineidae	0.019

Abbreviations: EPFU, big brown bat (*Eptesicus fuscus*); MYLU, little brown bat (*Myotis lucifugus*).

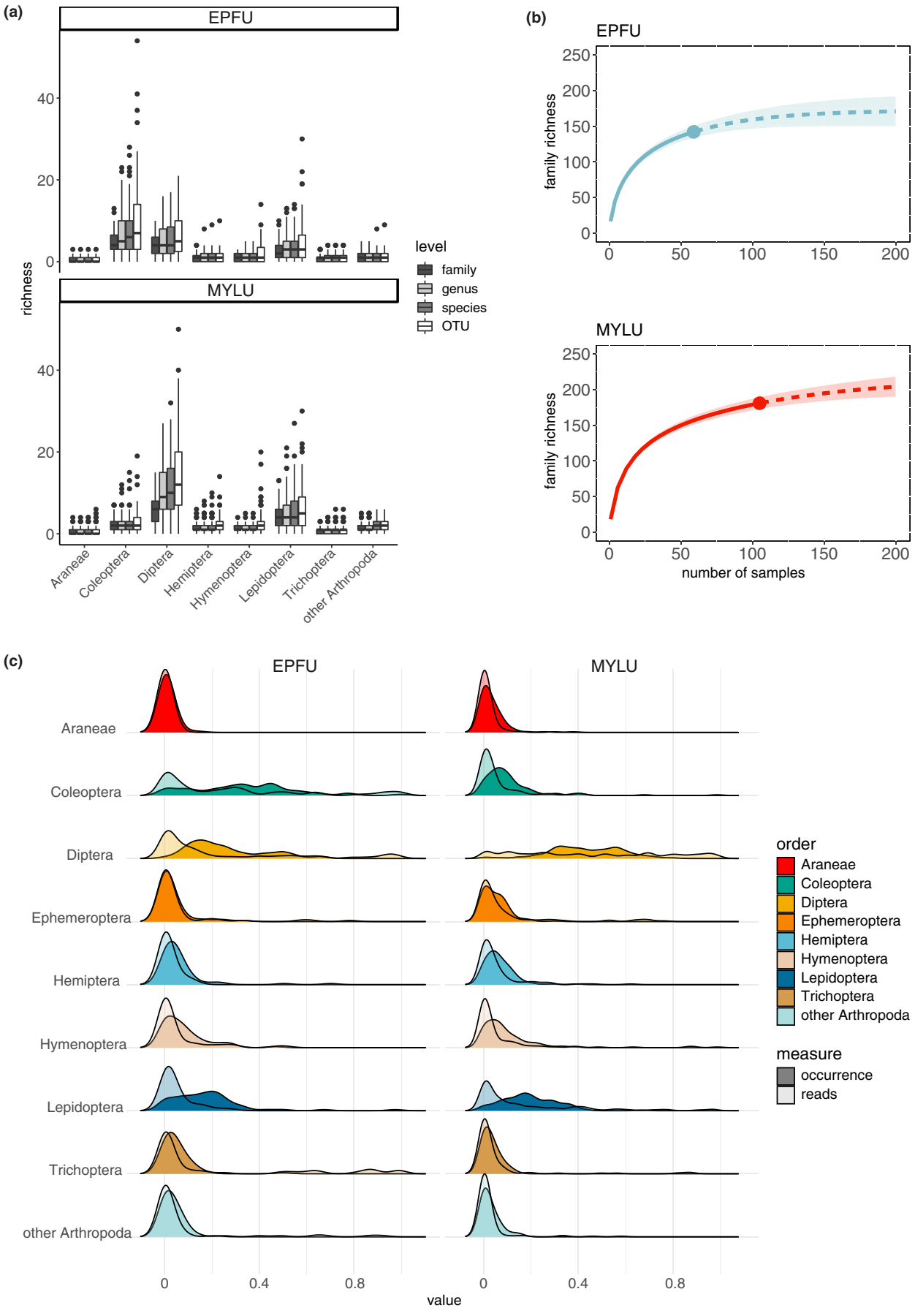
(a)
EPFU
MYLU
richness
level
family
genus
species
OTU
Araneae
Coleoptera
Diptera
Hemiptera
Hymenoptera
Lepidoptera
Trichoptera
other Arthropoda
(b)
EPFU
MYLU
family richness
number of samples
(c)
EPFU
MYLU
Araneae
Coleoptera
Diptera
Ephemeroptera
Hemiptera
Hymenoptera
Lepidoptera
Trichoptera
other Arthropoda
value
order
measure
occurrence
reads

FIGURE 1 Characterization of bat diets using HTAS. (a) Comparison of within-order richness at family, genus, species and OTU taxonomic levels. Black bar represents the median, boxes represent the interquartile range (IQR), whiskers represent minimum and maximum values, and shades indicate the taxonomic level for each major arthropod order. (b) Interpolated and extrapolated accumulation curves for family-level taxonomic richness. Solid lines represent interpolation, dotted lines represent extrapolation, and colours indicate bat species. (c) Density distribution of relative read abundance, with colours indicating major arthropod orders. Transparent colours represent RRA, a read-based metric of relative abundance within a sample, while solid colours represent wPO, a presence-based metric of relative abundance within a sample. EPFU, big brown bat (*Eptesicus fuscus*), MYLU, little brown bat (*Myotis lucifugus*)

cumulatively and for samples aggregated by the time period of sample collection and by collection site.

In order to test the influence of arthropod abundance on dietary variation, we conducted a constrained ordination using the R package 'vegan' (Oksanen et al., 2019). Specifically, we performed a redundancy analysis (RDA) separately for each bat species on presence/absence matrices at the family and OTU levels. RDA scores were extracted, and linear explanatory variables (including week, year and arthropod abundances at the local and regional scales) were then fit onto the ordination as environmental vectors using the 'envfit' function. For these analysis, local abundance represents the abundance of an arthropod group in a black-light sample corresponding to a guano sample from the same site and week, while regional abundance represents the mean abundance of an arthropod group aggregated across sites for each week. All analyses were conducted in R (R Core Team, 2020) with additional R packages used for data processing and visualization including 'dplyr', 'tidyverse', 'ggplot2', 'reshape2' and 'wesanderson' (Ram & Wickham, 2018; Wickham, 2020; Wickham, Chang, et al., 2020; Wickham, Francois, et al., 2020; Wickham et al., 2019).

3 | RESULTS

3.1 | Sequencing results

A total of 105 little brown bat samples (62.5%, n = 168) and 59 big brown (62.8%, n = 94) bat samples were successfully amplified. A total of 1,594 arthropod OTUs were detected in bat samples: 1,199 in little brown bat samples (75.2%) and 735 in big brown bat samples (46.1%). A total of 340 OTUs were detected in both bat species (21.3%). For little brown bats, 923 OTUs were identified to the family level (77.0%), 798 to the genus level (66.6%) and 618 to the species level (51.5%). For big brown bats, 540 OTUs were identified to the family level (73.5%), 496 to the genus level (67.5%) and 418 to the species level (56.9%). Between little brown and big brown bats, there was not a statistically significant difference in the number of OTUs identified at different taxonomic levels (χ^2 = 6, df = 4, p = .199). For both bat species, Hymenoptera and Araneae had the lowest percentages of OTUs identified beyond order, while Ephemeroptera had the highest percentages of OTUs identified beyond order (Table S1). For the insect mock community, all 34 known arthropods were recovered and identified. Our mock community includes 2 mock members that have known sequence variants that are included in the mock (Jusino et al., 2019), and those variants cluster with the originating sequence. Three additional OTUs were also detected, for which two were identified as separate variants of a known mock community member (*Apis mellifera*), and the other was detected at very low reads (n = 3 reads) and likely represents a chimeric sequence (Table S2).

3.2 | Bat diet composition

The most commonly detected OTUs and families for each bat species, as measured by incidence, wPO, and RRA, are reported in Tables 2a and 2b and 3, and in Table S3a–c. Among little brown bats, a significantly higher richness of Araneae, Diptera, Hemiptera and Lepidoptera families, genera and species was detected, while significantly fewer Coleopteran families, genera and species were detected in comparison with big brown bats (Figure 1a; Table S4). At the OTU level, a significantly higher richness of Araneae, Diptera, Hemiptera and other Arthropoda and significantly lower Coleoptera richness were detected among little brown bat samples (Figure 1a; Table S4). There was no statistically significant interspecific difference in the richness of Ephemeroptera, Hymenoptera or Trichoptera at any of the taxonomic levels. A total of 181 and 142 arthropod families were detected and identified in little brown and big brown bat samples, respectively. Using an asymptotic estimate of total family richness, 217 total families were predicted to be detected among little brown bat samples (95% CI = 198, 259), and 173 families were predicted to have been detected among big brown bat samples (95% CI = 156, 210) (Figure 1b). For little brown bat samples at the ordinal level, Diptera had the highest mean wPO and RRA (Figure 1c). Lepidoptera had the next highest mean wPO, while Coleoptera had the next highest mean RRA. For big brown bats at the ordinal level, Coleoptera had the highest mean wPO and RRA, while Diptera had the next highest mean wPO and mean RRA (Figure 1c).

3.3 | Arthropod abundance in black-light traps

Across all black-light samples, Trichoptera had the highest mean abundance ($\bar{x}$ = 645.4, IQR = 103.5–743.2), followed by Diptera: Culicidae/Chironomidae ($\bar{x}$ = 594.5, IQR = 16–222) and other Coleoptera ($\bar{x}$ = 243.3, IQR = 13.3–236.8; Figure 2a). The same groups also had the highest mean percentages of the total sample abundance, with Trichoptera representing 21.6% of the total sample abundance on average (IQR = 10.7–37.2%), followed by Diptera: Culicidae/Chironomidae ($\bar{x}$ = 10.3, IQR = 2.0–10.3%) and

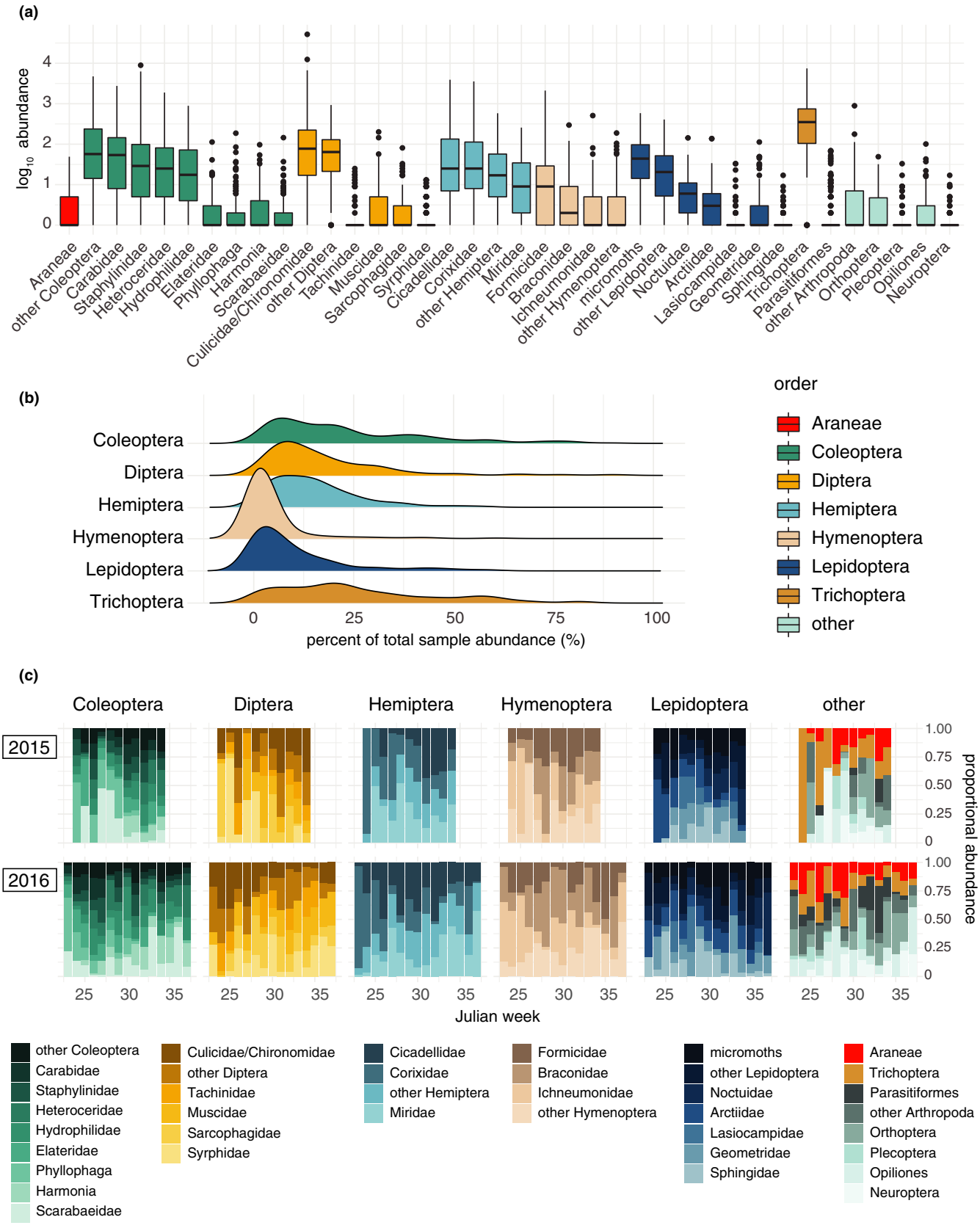

FIGURE 2 Characterizing arthropod prey communities using black-light traps. (a) Log_{10} abundance of focal arthropod groups in black-light traps. Black bar represents the median, boxes represent the interquartile range (IQR), whiskers represent minimum and maximum values, and colours indicate major arthropod orders. (b) Density distribution of the percentage of total sample abundance for major arthropod orders as a per cent of the total arthropod abundance in black-light trap samples. (c) Black-light trap intra-ordinal community composition by Julian week in years 2015 and 2016. Colours represent major arthropod orders and groups, and the shades of each colour represent lower-level taxonomic groups within each category

other Coleoptera ($\bar{x}$ = 7.9, IQR = 1.7–10.0%) When grouped by orders, the highest mean percentages of total sample abundance were Coleoptera ($\bar{x}$ = 23.2, IQR = 7.8–33.6%), Trichoptera and Diptera ($\bar{x}$ = 17.4, IQR = 7.3–22.1%). Between years, there were significantly lower raw abundances of total Hemiptera ($t_{96.86}$ = 2.64, p = .01), Hymenoptera ($t_{58.91}$ = 2.09, p = .04) and Lepidoptera ($t_{52.44}$ = 4.60, p < .001) in 2016. Qualitatively, prey communities were seldom dominated by any one particular taxonomic group (Figure 2b), although groups were highly variable overall and changed from week to week (Figure 2c).

3.4 | Relating dietary composition with arthropod abundance

3.4.1 | Big brown bats (*Eptesicus fuscus*): logistic regression

For big brown bats, the model including collection site as variable performed significantly better than the null model (p < .001) and was therefore retained as a predictor variable. For this analysis, 7 arthropod groups had a statistically significant positive main effect on the probability of detection in diet and 8 arthropod groups had a statistically significant negative main effect on the probability of detection in diet (Figure 3a). None of the groups had a statistically significant interaction with its respective abundance after accounting for the main effect of arthropod group identity (Figure 3b).

3.4.2 | Little brown bats (*Myotis lucifugus*): logistic regression

For little brown bats, the model including Julian week as a variable performed significantly better than the null model (p = .007) and was therefore retained as a predictor variable. For this analysis, 3 arthropod groups had a statistically significant positive main effect on the probability of detection in diet and 17 arthropod groups has a statistically significant negative main effect on the probability of detection in diet (Figure 3a). Corixidae, other Hemiptera, other Lepidoptera and Trichoptera had a marginally significant interaction with abundance (p = .015, β_{int} = 1.004, OR = 0.308; p = .020, β_{int} = 0.785, OR = 0.609; p = .042, β_{int} = 0.838, OR = 1.96; p = .037, β_{int} = 0.438, OR = 1.11; Figure 3b).

3.4.3 | Diversity metrics

Big brown bats had higher rates of turnover than little brown bats for all samples overall and for samples aggregated by time and collection site, which was consistent for all orders of diversity and for both RRA and wPO (Table 4). For both bat species, turnover aggregated by site was also higher than turnover aggregated by time period (Table 4). We found no relationship between the Hill numbers calculated from bat guano samples and the Hill numbers calculated from corresponding black-light trap samples, which was consistent for all orders of diversity (Figure 3c).

3.4.4 | Influences of local and regional abundances on dietary composition

For big brown bats at the family level, local Coleoptera and local Lepidoptera abundances were significant vectors in the ordination (R^2 = .197, p = .012; R^2 = .156, p = .029), while local Hemiptera, local Hymenoptera and regional Hemiptera were marginally significant vectors (R^2 = .122, p = .057; R^2 = .101, p = .100; R^2 = .124, p = .085; Figure 4a). For big brown bats at the OTU level, week and local Lepidoptera abundances were marginally significant vectors (R^2 = .161, p = .065; R^2 = .154, p = .079; Figure 4b). For little brown bat diets at the family level, local Hymenoptera, local Trichoptera, regional Hemiptera and regional Trichoptera abundances were significant vectors (R^2 = .121, p = .038; R^2 = .112, p = .029; R^2 = .108, p = .048; R^2 = .111, p = .047), while week, local Hemiptera and local total abundances were marginally significant vectors (R^2 = .094, p = .057; R^2 = .100, p = .055; R^2 = .081, p = .084; Figure 4a). For little brown bats at the OTU level, local Coleoptera, local Diptera, local Trichoptera, local total and regional Trichoptera abundances were significant vectors (R^2 = .323, p = .001; R^2 = .277, p = .001; R^2 = .170, p = .005; R^2 = .306, p = .001; R^2 = .146, p = .016), while regional Diptera and regional total abundances were marginally significant vectors (R^2 = .095, p = .078; R^2 = .104, p = .061; Figure 4b).

4 | DISCUSSION

The results from this study support our hypothesis that generalist predators would display preferences for certain prey and that the local abundance of a prey group would not strongly influence the probability of its consumption. Although some less commonly consumed groups were slightly more likely to be consumed when they were more abundant, the statistical magnitude of these effects was generally small. We also found no evidence of correlation between dietary diversity and underlying prey diversity. Among both bat species, we found that prey abundance influenced community-level dietary composition, suggesting that bats do adjust their foraging patterns in response to changing prey resources, though not necessarily as a direct response to increasing quantitative abundance of a particular prey resource. As the dietary data resulting from HTAS cannot necessarily be extrapolated to represent prey quantities (Brandon-Mong et al., 2015; Clarke et al., 2014; Piñol et al., 2015), our results are not a true estimation of a functional response. Nonetheless, as described below, this study provides insights into how changes in prey abundance affect the probability of prey consumption and the overall dietary composition in two highly generalist predators.

4.1 | Relating prey detection in bat diets with arthropod abundance

Quantifying prey availability is difficult for generalists that consume hundreds or even thousands of prey items. All arthropod trapping methods carry biases and do not necessarily sample arthropod communities evenly (Kirkeby et al., 2013; Kremen et al., 1993). In this study, we characterized arthropod communities by comparing the raw abundance of groups, the percentage of total sample abundance and the intra-order proportional abundance of each group. Overall, we found that the night-flying arthropod communities in this study system were highly variable, but seldom dominated by a single arthropod group. Trichoptera were consistently abundant, as were certain groups within Diptera (namely, Culicidae/Chironomidae and other Diptera) and within Coleoptera (namely Carabidae, Staphylinidae and other Coleoptera). Our results suggest that prey communities in this study system are generally not characterized by large resource pulses, at least among the taxa that are well represented by the arthropod trapping method. Despite the many challenges in relating prey abundance with generalist dietary composition, by sampling arthropod abundance near bat roosts and comparing the relative abundance of each group with the probability of its detection in guano samples, our study represents one of the most intensive efforts to associate quantitative prey information with a noninvasive HTAS-based diet study.

Perfectly sampling the entire suite of prey available to a colony of bats is impossible given large home and foraging range sizes, the diversity of available prey, and the range of different habitats those prey occupy. In this study, bats were observed flying near black-light trap locations during roost emergence counts, and as part of a separate study, passive acoustic monitoring indicated that bat foraging activity was high near black-light trap sampling locations (Wray et al. unpublished data). Moreover, lactating female little brown bats have been shown to usually forage within 600 m of the roost (Henry et al., 2002). Thus, we conclude that there is a reasonable a priori expectation that bat diets could track spatiotemporal variation in arthropods present at arthropod sampling locations. Nevertheless, we acknowledge that our sampling design may not fully reflect arthropod communities for bats with large foraging ranges and we suggest that future studies could also incorporate tracking efforts (perhaps in nonbreeding bats or in populations that are not currently threatened) or could conduct sampling in multiple habitat types at various distances from bat roosts. We also acknowledge that black-light trap samples cannot capture the total spectrum of prey available for a highly mobile predator. The data resulting from arthropod communities as captured by black-light traps and prey communities present in diets as detected by HTAS are difficult to compare, and subsequent research efforts may consider incorporating additional HTAS analyses for prey communities. However, such studies would still require some measure of quantitative prey abundance measurement through trapping or survey efforts, since HTAS data are semi-quantitative (Deagle et al., 2019; Jusino et al., 2019; Palmer et al., 2018). Using HTAS for both prey and diet communities would also necessitate additional measures (such as processing in separate laboratories) to avoid issues with contamination and may require further evaluation of potential amplification biases between prey community samples and faecal samples due to differences in template quality.

Classical measures of preference dictate that determining which prey are preferred requires information on both prey consumption and prey abundance, availability or density (Chesson, 1978, 1983; Rapport & Turner, 1970). In this study, we found that after incorporating prey abundance, the interaction between prey group identity and prey abundance was not statistically significant for most prey groups, though the magnitude of the statistical effect size of prey group identity was influenced by abundance. For example, the effect size of the highest ranked categories for both bat species based on diet information alone decreased slightly after accounting for their respective abundance (Figure S2). Among both bat species, although several different prey groups had the largest effect sizes based on diet alone, other Diptera had the largest effect size when including abundance. These results, however, could be an artefact of either the grouping of Diptera taxa or the low abundance of Limoniidae in black-light trap samples. Alternatively, among little brown bats, both Chironomidae (in the model with diet only) and the group combining Chironomidae and Culicidae (in the model with diet and abundance) maintained large effect sizes, although the Chironomidae/Culicidae group was among the most abundant arthropod groups present in black-light trap samples. Overall, the results from this study demonstrate that while prey identity generally appears to outweigh abundance in determining the probability of detection in bat diets, incorporating some measures of background prey abundance

FIGURE 3 Relationships between bat diets and local arthropod prey abundance and diversity. (a) Binary logistic regression main effects of arthropod group identity as predictors of the probability of detection (presence/absence) of arthropod prey in bat guano samples. Points indicate the estimate, and lines indicate the 95% confidence interval. The dotted line indicates zero, such that confidence intervals nonoverlapping with zero suggest statistically meaningful model terms. Closed circles indicate overlap with zero, open triangles indicate nonoverlap with zero. (b) Binary logistic regression interaction effects between arthropod group identity and quantitative arthropod abundance as predictors of the probability of detection in guano samples after accounting for the main effect of group identity. Points indicate the estimate, and lines indicate the 95% confidence interval. The dotted line indicates zero, such that confidence intervals nonoverlapping with zero suggest statistically meaningful model terms. Closed circles indicate overlap with zero, open triangles indicate nonoverlap with zero. (c) Generalized linear model (GLM) regression of Hill numbers in black-light trap samples as predictors of Hill numbers in bat guano samples, estimated at different orders of diversity measures (*q*). EPFU = big brown bat (*Eptesicus fuscus*), represented by light blue circles in panel C; MYLU = little brown bat (*Myotis lucifugus*), represented by red triangles in panel C

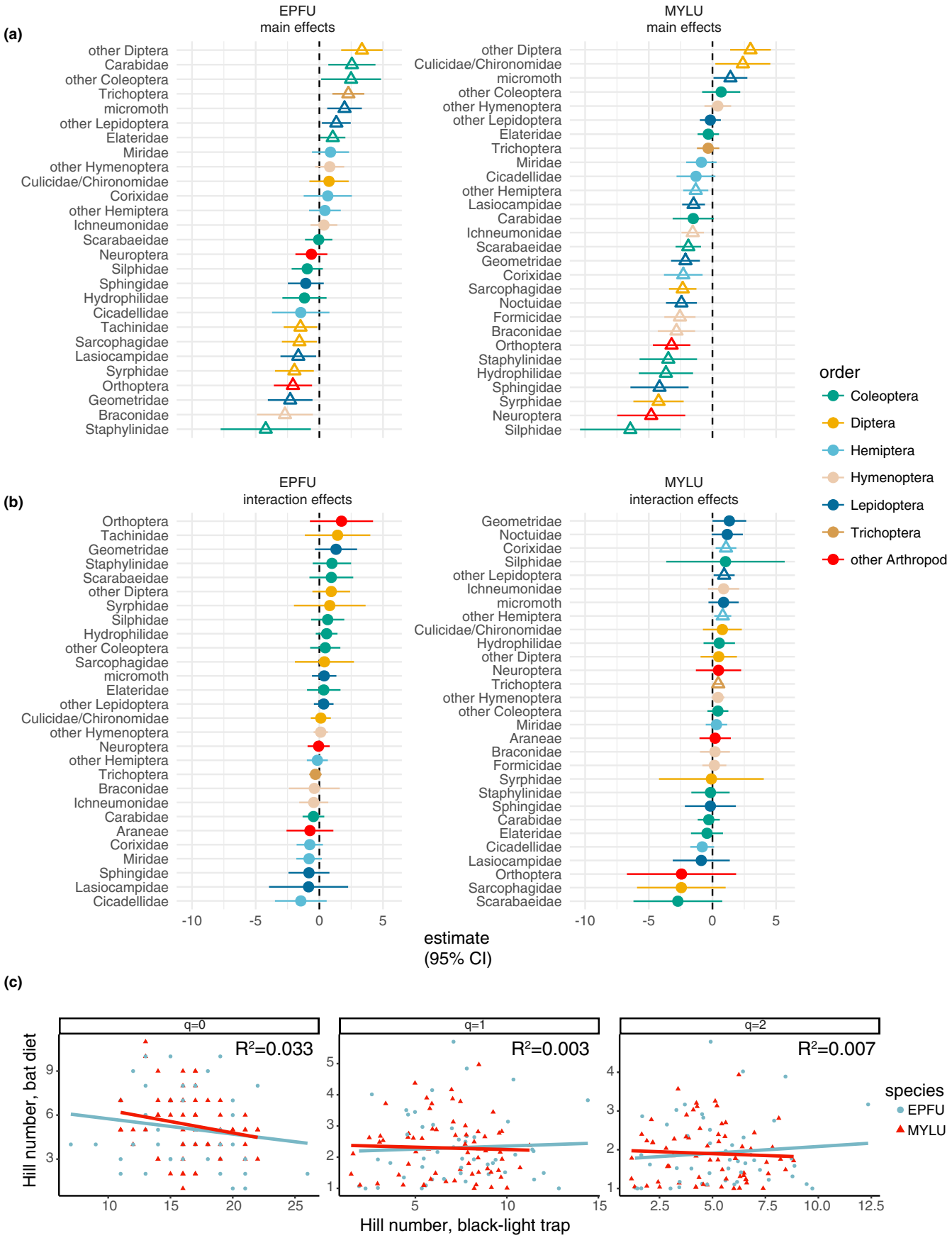
(a)
EPFU
main effects
other Diptera
Carabidae
other Coleoptera
Trichoptera
micromoth
other Lepidoptera
Elateridae
Miridae
other Hymenoptera
Culicidae/Chironomidae
Corixidae
other Hemiptera
Ichneumonidae
Scarabaeidae
Neuroptera
Silphidae
Sphingidae
Hydrophilidae
Cicadellidae
Tachinidae
Sarcophagidae
Lasiocampidae
Syrphidae
Orthoptera
Geometridae
Braconidae
Staphylinidae
MYLU
main effects
other Diptera
Culicidae/Chironomidae
micromoth
other Coleoptera
other Hymenoptera
other Lepidoptera
Elateridae
Trichoptera
Miridae
Cicadellidae
other Hemiptera
Lasiocampidae
Carabidae
Ichneumonidae
Scarabaeidae
Geometridae
Corixidae
Sarcophagidae
Noctuidae
Formicidae
Braconidae
Orthoptera
Staphylinidae
Hydrophilidae
Sphingidae
Syrphidae
Neuroptera
Silphidae
order
Coleoptera
Diptera
Hemiptera
Hymenoptera
Lepidoptera
Trichoptera
other Arthropod
(b)
EPFU
interaction effects
Orthoptera
Tachinidae
Geometridae
Staphylinidae
Scarabaeidae
other Diptera
Syrphidae
Silphidae
Hydrophilidae
other Coleoptera
Sarcophagidae
micromoth
Elateridae
other Lepidoptera
Culicidae/Chironomidae
other Hymenoptera
Neuroptera
other Hemiptera
Trichoptera
Braconidae
Ichneumonidae
Carabidae
Araneae
Corixidae
Miridae
Sphingidae
Lasiocampidae
Cicadellidae
-10
-5
0
5
MYLU
interaction effects
Geometridae
Noctuidae
Corixidae
Silphidae
other Lepidoptera
Ichneumonidae
micromoth
other Hemiptera
Culicidae/Chironomidae
Hydrophilidae
other Diptera
Neuroptera
Trichoptera
other Hymenoptera
other Coleoptera
Miridae
Araneae
Braconidae
Formicidae
Syrphidae
Staphylinidae
Sphingidae
Carabidae
Elateridae
Cicadellidae
Lasiocampidae
Orthoptera
Sarcophagidae
Scarabaeidae
-10
-5
0
5
estimate
(95% CI)
(c)
q=0
R²=0.033
q=1
R²=0.003
q=2
R²=0.007
Hill number, bat diet
Hill number, black-light trap
species
EPFU
MYLU

TABLE 4 Family-level Sørensen turnover rates of read-based (RRA) and incidence-based (wPO) bat diet composition for all samples and for samples aggregated by collection site and time (i.e. the week and year of sample collection).

RRA					wPO				
		q=0	*q*=1	*q*=2			*q*=0	*q*=1	*q*=2
All samples	EPFU	0.166	0.112	0.095	All samples	EPFU	0.166	0.068	0.047
	MYLU	0.108	0.074	0.057		MYLU	0.108	0.039	0.018
Site	EPFU	0.310	0.220	0.248	Site	EPFU	0.310	0.113	0.058
	MYLU	0.197	0.185	0.184		MYLU	0.197	0.081	0.038
Time	EPFU	0.207	0.149	0.167	Time	EPFU	0.207	0.097	0.082
	MYLU	0.139	0.099	0.083		MYLU	0.139	0.057	0.033

The order of the diversity measure is represented by *q*.

Abbreviations: EPFU, big brown bat (Eptesicus fuscus); MYLU, little brown bat (Myotis lucifugus).

remains important for accurately estimating the preferences of a predator.

Despite potential limitations in estimating prey availability, our results provide strong evidence that changes in local prey abundance have little effect on the probability of prey detection in bat diets. As a notable exception, other Lepidoptera (a group representing Lepidoptera not belonging to the focal groups of the study or not identified beyond the ordinal level) were more likely to be detected in little brown bat guano samples as a function of increasing abundance. Previous morphological studies have demonstrated that little brown bats can switch between opportunistic and selective foraging depending on seasonality and reproductive stage (Anthony & Kunz, 1977; Belwood & Fenton, 1976; Burles et al., 2008). Indeed, the focal species of this study are highly mobile predators in a complex system with many alternative prey resources, and thus, their responses to changing resource availability are difficult to predict without corresponding foraging movement information such as radio-tracking data (e.g. Almenar et al., 2013). Bats have also been documented employing opportunistic foraging around objects, such as lights and even animals, that attract arthropods (Palmer et al., 2019; Rowse et al., 2016), suggesting the possibility that black-light traps may have an effect on bat foraging or on prey community sampling. It is also important to note that certain taxa (such as Ephemeroptera and Diptera: Limoniidae) were frequently detected in bat diets but seldom captured in black-light traps, likely because they are not particularly attracted to the type of trap used in this study.

While prey abundance was generally unrelated to the probability of consumption, we found that both local and regional abundances had influences on the community-level dietary composition of both bat species. These results varied slightly depending on the taxonomic levels that were assessed. For big brown bats, OTU-level dietary composition appeared to be less influenced by temporal factors or arthropod group abundance, while family-level dietary composition appeared to be more strongly influenced by the local or regional abundance of several groups. In contrast, little brown bat dietary composition was influenced by combinations of local and regional arthropod abundances, many of which were consistent at both taxonomic levels. The effect of Julian week, in contrast, appeared to have an effect only on the family-level community composition. These results suggest several processes, particularly in the light of measurements of turnover. First, the dietary composition of big brown bats had higher overall turnover, despite displaying less clear responses to changing arthropod abundance. However, the overall arthropod community sampling results from this study suggest that big brown bats tend to consume prey that are intrinsically less variable in quantitative abundance. Second, turnover calculations indicated that the dietary composition of both bat species was more similar when aggregated by site than when aggregated by time, indicating that temporal changes may be more influential than local-scale spatial changes in prey availability.

Our findings demonstrate that little brown and big brown bats can adjust their foraging strategies in response to changes in prey communities, but that the probability of detecting prey in their diets does not increase directly as a function of quantitative prey availability and likely involves complex behaviours related to prey preferences. Despite interspecific differences in total dietary composition, both bat species displayed strong preferences for particular prey. These patterns are consistent with previous studies, suggesting that these bat species are usually not limited by prey availability and do not compete directly with each other, likely due to their physiological differences and high dispersal abilities (Barclay & Brigham, 1991; Kunz, 1973; Moosman et al., 2012). We also found that arthropod predators such as spiders, predatory beetles and lacewings were somewhat common in the diets of both bat species. In combination with their apparent selectivity, foraging at a high trophic level suggests that these bat species could have both consumptive and nonconsumptive effects on arthropod communities, which may consequently alter prey behaviour or otherwise complicate the relationship between prey availability and prey consumption. The patterns observed in this study may also be influenced by some degree of individual-level specialization (Bolnick et al., 2002), as both little brown and big brown bats tend to have large maternity colonies (Fenton, 1980; Kurta & Baker, 1990), and the sampling design of this study represents colony-level diet composition. Overall, our results provide additional evidence that selective predation among

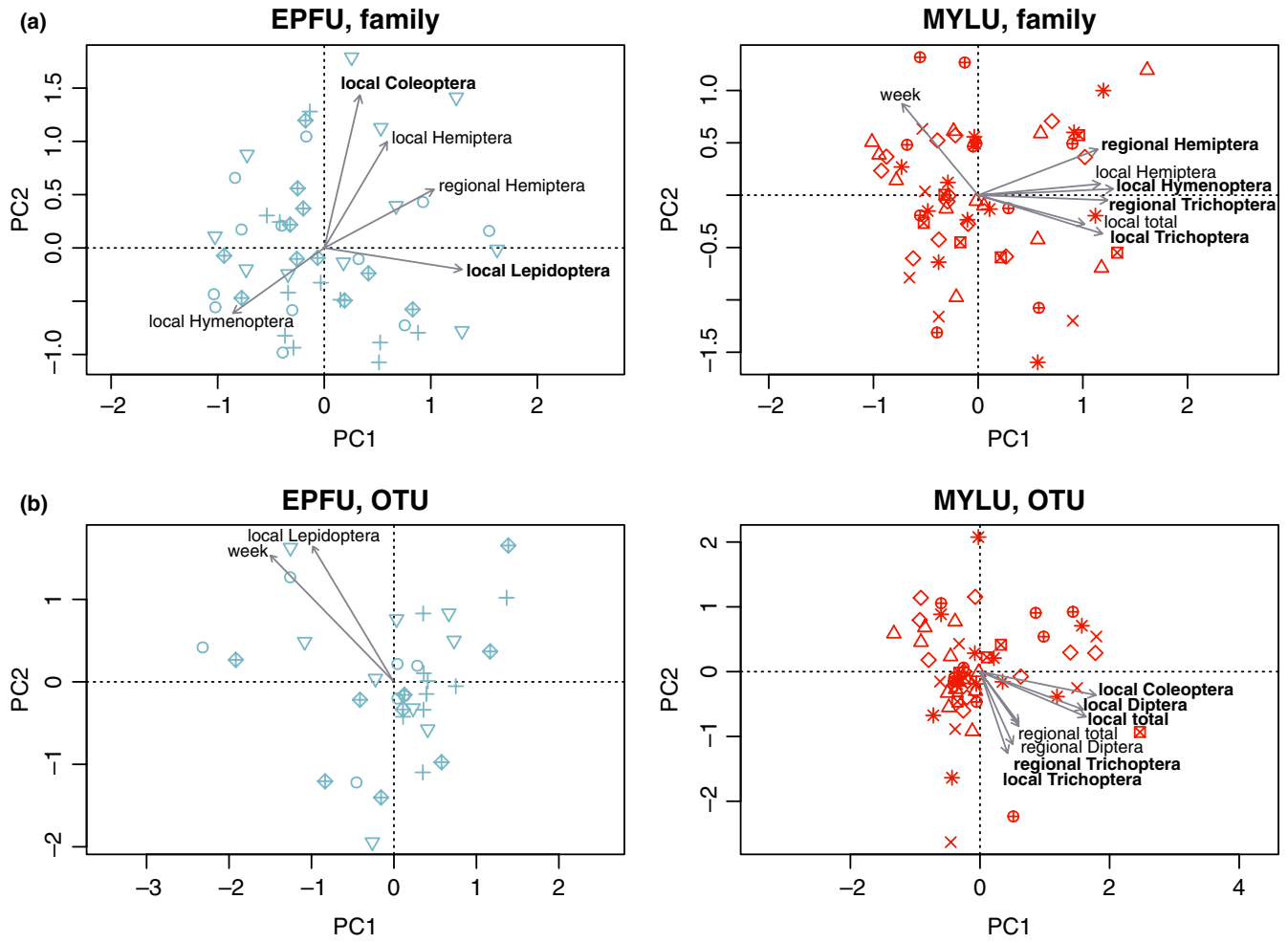

FIGURE 4 Influences of temporal variables and arthropod abundance on the composition of bat diets. Local abundance represents the abundance of arthropod groups at a site in a particular week and year, while regional abundances represent the abundance of arthropod groups at all sites in a particular week and year. (a) Redundancy analysis (RDA) plots based on family-level presence/absence matrices, with overlaid statistically significant and marginally significant environmental vectors. (b) RDA plots based on OTU level presence/absence matrices, with overlaid statistically significant and marginally significant environmental vectors. Bold text indicates environmental vectors with $p < .05$, while regular text indicates environmental vectors with $p < .10$. Point symbols represent distinct sampling sites. EPFU, big brown bat (*Eptesicus fuscus*), MYLU, little brown bat (*Myotis lucifugus*)

generalists may be more common than previously thought, particularly among predators that are highly mobile and that forage in species-rich systems.

4.2 | Implications for HTAS studies on predators of arthropods

The diets of both bat species contained many taxonomic groups, but Diptera and Coleoptera had the highest OTU, species, genus and family richness among little brown and big brown bat guano samples, respectively. A higher taxonomic richness of prey items was detected in little brown bat diets in comparison with big brown bat diets, with accumulation curves indicating that sample sizes in this study were sufficient for drawing comparisons between bat species. These results are generally consistent with previous studies that used both molecular and morphological methods (e.g, Agosta, 2002; Anthony & Kunz, 1977; Belwood & Fenton, 1976; Burles et al., 2008; Clare, Symondson, & Broders, 2014; Clare, Symondson, & Fenton, 2014). Notably for both bat species, the percentage of OTUs identified to the species, genus and family levels were highly variable within different arthropod orders. For example, while Diptera: Chironomidae had the highest richness of OTUs, this family is highly speciose and well represented in reference databases. Despite ever-increasing database building efforts, arthropods still tend to have fewer reference sequences identified beyond the ordinal level, and often retain incomplete or unresolved taxonomy (Hebert et al., 2016; Stork, 2018). Thus, using HTAS for dietary studies in a highly generalist predator that consumes prey from underrepresented taxonomic groups represents a unique challenge from several perspectives.

While the taxonomic richness of prey items can serve as a proxy of underlying functional or genetic diversity, read-based and presence-based metrics (e.g. RRA and wPO, respectively) are also

frequently used for characterizing dietary composition. In this study, weighted presence-based and read-based measures were generally consistent, with a few exceptions. For example, Lepidoptera tended to have a mean RRA that was much lower than the mean wPO for both bat species. Similarly, the mean RRA for Diptera tended to be lower than the mean wPO for big brown bats. These differences may be attributed to biases inherent to occurrence-based metrics, which can potentially overestimate the importance of food items consumed in low quantities and can be highly sensitive to contamination issues (Deagle et al., 2019; Lamb et al., 2019). In contrast, we found that among big brown bat guano samples an OTU assigned to *Potamyia flava* (Trichoptera: Hydropsychidae) had a mean RRA that was more than three times higher than the OTU with the next highest mean RRA. The same OTU was also detected among little brown bat guano samples, but did not have an unusually high mean RRA, and other members of the mock community in the order Trichoptera did not have unusually high reads (Figure S3). Additionally, an evaluation of the primer set used in this study showed that other frequently used primers (ZBJ, COI L/H) did not detect *P. flava* (Jusino et al., 2019). The high mean RRA of this prey item among big brown bat guano samples could also be driven by instances where few total prey items were detected. However, other studies have noted that Trichoptera, which often emerge en masse, may be particularly desirable to bats (Whitaker, 2004). In the context of the mock community and ecological background information, the read-based metrics associated with *Potamyia flava* in big brown bat diets could potentially reflect some degree of biomass within guano samples. Although read-based metrics can be highly sensitive to recovery and PCR biases, and as such, their value remains only semi-quantitative (Deagle et al., 2019; Jusino et al., 2019; Palmer et al., 2018), these results nonetheless demonstrate the utility of mock communities for comparing and contextualizing both read-based and presence-based metrics.

When comparing our results with previous morphological and molecular studies, the importance of defining taxonomic levels was readily apparent. For example, we found that for both bat species, the OTU with the highest raw incidence did not belong to the family with the highest raw incidence. Similarly, for big brown bats the OTU with the highest mean wPO and mean RRA at the OTU level corresponded to the highest family-level mean RRA, but not to the family-level mean wPO. In contrast, for little brown bats, the OTU with the highest mean RRA did not correspond to the highest family-level mean RRA or wPO. These results suggest that, in addition to differences between richness-based, read-based and presence-based metrics, considering the taxonomic level of prey detected in dietary samples can also influence the interpretation of HTAS data. Strategies such as aggregating prey categories at higher taxonomic levels or assigning trait-based functional analyses may provide better approximations of prey resource states (e.g. Arrizabalaga-Escudero et al., 2019), as the high resolution of most OTU-based prey categories likely do not correspond to how prey are actually distinguished by predators.

Comparing dietary composition with prey availability in many highly generalist species, including arthropodivorous vertebrates, remains challenging, particularly when connecting the different data types resulting from both molecular methods and capture-based studies. However, as this study demonstrates, the most frequently consumed prey and the preferred prey are not necessarily the same, and some measure of underlying prey availability must be quantified in order to accurately determine when predation is selective. The need for improved practices among DNA barcoding for dietary studies has been highlighted by several recent papers (e.g. Alberdi et al., 2019; Jusino et al., 2019; Zinger et al., 2019). However, comparatively fewer studies have provided guidelines for the interpretation of data in terms of understanding ecological processes. While we encourage the use of robust positive controls—such as mock communities—as a solution for parameterizing the biases inherent to molecular methods, we also emphasize the serious need for considering how the resulting data can be interpreted in order to fit within an ecological framework.

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AUTHOR CONTRIBUTIONS

MZP, CG and DLL designed the study. AKW and JMK performed field and laboratory work. MAJ provided the arthropod mock community. MAJ, MTB, JMP and AKW conducted sequencing and bioinformatics processing. AKW analysed the data and created figures. AKW and MZP wrote the manuscript. All authors reviewed and edited the manuscript.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in SRA at https://dataview.ncbi.nlm.nih.gov/object/PRJNA668526?reviewer=rfk77a9krsl4d12chvpl19sh7d. Accession no. PRJNA668526.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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