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# Eating local: influences of habitat on the diet of little brown bats (*Myotis lucifugus*)

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## Abstract

**We employ molecular methods to profile the diet of the little brown bat, *Myotis lucifugus*, and describe spatial and temporal changes in diet over their maternity season. We identified 61 prey species of insects and 5 species of arachnid. The largest proportion of prey (~32%) were identified as species of the mass-emerging Ephemeroptera (mayfly) genus *Caenis*. Bats roosting in agricultural settings had lower dietary richness than those occupying a roost located on a forest fragment in a conservation area. We detected temporal fluctuations in diet over the maternity season. Dipteran (fly) species dominated the diet early in the season, replaced later by species of mayfly. Because our methodology provides species-level identification of prey, we were able to isolate environmental indicator species in the diet and draw conclusions about the location and type of their foraging habitat and the health of these aquatic systems. The species detected suggested that the bats use variable habitats; members of one agricultural roost foraged on insects originating in rivers or streams while those in another agricultural roost and the forest roost fed on insects from pond or lake environments. All source water for prey was of fair to good quality, though no species detected are intolerant of pollution thus the habitat cannot be classified as pristine. Our study outlines a model system to investigate the abiotic and biotic interactions between habitat factors through this simple food chain to the top predator.**

*Keywords*: community ecology, indicator species, predator–prey interactions, resource use, species' interactions

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## Introduction

Species' interactions underlie ecosystem functioning and understanding their patterns is crucial for predicting their responses to disturbance. For many taxa, these relationships are poorly documented (Sheppard *et al.* 2004) and this is particularly true of insectivorous bats which hunt at night, often in areas where observation is impossible. Traditional dietary analysis of insectivores relies on morphological classification of post-digested prey fragments in faeces (guano) (Kunz & Whittaker 1983) but rapid and thorough chewing and digestion by bats ensures that these techniques rarely provide identifications beyond order or family (e.g. Kunz 1974; Belwood & Fenton 1976; Kunz & Whittaker 1983). Because species-level identification of most prey is nearly impossible, and small soft-bodied insects are often missed entirely (Kunz & Whittaker 1983), inferences about dietary variation and predator preferences are severely constrained by traditional methods.

Recent advances in molecular analysis have made it possible to identify prey species from trace material containing DNA fragments (reviewed by Symondson 2002; King *et al.* 2008) in a variety of invertebrate (e.g. Coulson *et al.* 1990; Zaidi *et al.* 1999; Kasper *et al.* 2004; Blankenship & Yayanos 2005) and vertebrate (e.g. Jarman *et al.* 2002; Jarman & Wilson 2004; Carter *et al.* 2006; Clare *et al.* 2009) predators. Many molecular techniques have been advanced to detect a small range of

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prey taxa (e.g. monoclonal antibodies) or multiple prey with several molecular targets (e.g. Scribner & Bowman 1998). While effective, these can be costly and analytically challenging, requiring different amplification systems and a priori knowledge of anticipated prey to pick the correct molecular target. More recently, single-marker DNA systems with more widespread prey detection ability have been developed simplifying these investigations, and pyrosequencing techniques (e.g. Deagle *et al.* 2009) are becoming more common. For bats, Clare *et al.* (2009) employed a single-marker technique useful in identifying a wide variety of terrestrial invertebrate prey and applied this to analyse the diet of the eastern red bat, *Lasiurus borealis*. The technique recovers DNA from individual prey fragments rather than employing whole faecal extraction of guano pellets and provides the opportunity to identify a large variety of invertebrate (particularly insect) prey without a priori assumptions about identity. This approach is particularly useful because it preferentially isolates prey rather than predator DNA and, because it targets individual insect fragments, removes amplification biases encountered with PCR on mixed samples and does not require subsequent cloning (e.g. Zeale *et al.* 2011) to separate amplicons for sequencing.

Diet in many bat species varies through time and space: colonies in similar habitats eat similar prey while heterogeneous habitats cause differential prey consumption (Aldridge & Rautenbach 1987) and predatory behaviour (Rydell *et al.* 1996). Temporal availability and prey abundance affect diet (Rydell *et al.* 1996) and gender (Belwood & Fenton 1976) and age (Adams 1997) may also influence the predator-prey relationship. The little brown bat, *Myotis lucifugus*, is widespread in North America though many populations are now under threat of local extinction due to the spread of White Nose Syndrome (Blehert *et al.* 2009; Frick *et al.* 2010). Little brown bats hunt emerging insects along waterways. Using morphological techniques, Belwood & Fenton (1976) identified Diptera and Trichoptera as major dietary components while Lepidoptera and Coleoptera were detected in lower proportions, though more specific analysis of diet was not possible. Particularly now, with the threat of local extirpation of little brown bat populations (Frick *et al.* 2010), it is important to profile the dietary requirements of this predator in detail to (i) understand the habitat requirements of these populations for conservation purposes and (ii) to make predictions about which (if any) insect populations may respond to a sudden release from predation.

While spatial and temporal heterogeneity in insect availability influences diet, comparing dietary variation between colonies can, in turn, be used to make inferences about foraging habitat. Particularly in aquatic systems, many species are considered environmental indicators of habitat type and quality, and their appearance as dietary prey can provide information on the predator's foraging location. Bats, like *M. lucifugus*, which prey directly on insects emerging from water which are, in turn, impacted by aquatic health, provide a unique model system to study the influences of environmental health through the food chain: from abiotic characteristics to top predators.

In this study we employ the molecular methods of Clare *et al.* (2009) to profile the diet of *M. lucifugus* over their maternity season and test the predictions (i) that there are spatio-temporal changes in diet, and (ii) that comparing species consumed between colonies provides information on habitat type, quality and location.

## Materials and methods

### *Guano Pellet collection, dissection and genetic analysis*

We collected and froze guano accumulating on sheets placed under maternity roosts bi-weekly from May 6 to August 19 2008 at three sites in Southwestern Ontario – agricultural roost 1 (Clinton) and agricultural roost 2 (Norfolk County) and a forest roost (Richmond Hill) located on conservation land ($\sim$1 km$^2$ forest patch). We left the sheets for $\sim$7 days prior to each collection. Our monitoring was unbalanced due to differential establishment, identification and departure of the colonies. As such, we monitored agricultural roost 1 for a 6-week period and agricultural roost 2 for a 14-week period, both starting the first week of June. We monitored the forest roost (FR) from May 6 to August 19. As these roosts were estimated to host hundreds to thousands of bats, we collected up to 1 L of guano at each roost on each collection date. Due to the volume of guano collected, we removed a sample of guano pellets from each collection and crushed them in ethanol under a dissection microscope. From this crushed material, we removed prey fragments for genetic identification. We attempted to sample fragments from different guano pellets (see discussion on assumptions of sampling independence). For each bi-weekly collection, we analysed 96 prey fragments per site (in 96-well plates).

We conducted DNA extraction, amplification and sequencing of all samples at the Biodiversity Institute of Ontario, University of Guelph following the protocols for bat dietary analysis established by Clare *et al.* (2009). Our prey identification method followed Clare *et al.* (2009) which is similar to the 'strict' method of Ross *et al.* (2008) and employs both a sequence similarity (we use >98% identity) and phylogenetic approach to species identification using a taxonomically validated reference sequence database for comparison. This is a

conservative method but was chosen to minimize type one errors in species identification (e.g. there are cases of >2% sequence divergence within species at this locus, but in this study an unknown matching a reference sequence with <98% similarity is discarded as unidentified).

### *Dietary diversity and MOTU*

In addition to full taxonomic identification of prey we estimated the number of species consumed based on the genetic diversity of the recovered sequences. We employed Molecular Operational Taxonomic Units (MOTU) (Floyd *et al.* 2002; Floyd 2003) in the program jMOTU (Anisah Goorah, Martin Jones and Mark Blaxter, https://www.nematodes.org/bioinformatics/jMOTU/) which groups sequences by a user-defined boundary of similarity. In our case, MOTU were estimated at seven different base pair resolutions selected to approximate 1%, 1.5%, 2%, 2.5%, 3%, 3.5% and 4% sequence divergence of the sequenced region.

We compared mean dietary richness estimated from MOTU analysis between the two agricultural roosts and the forest roost. As sampling was unbalanced we compared only paired samples from the weeks of June 4–July 22. For these paired samples we also compared the proportion of un-identified taxa as the mean number of estimated MOTU minus actual identifications. For our temporal analysis we analysed data from the forest roost which was monitored for the longest period. We partitioned the data by date; early (May 6–June 15), mid (June 16–July 18) and late (July 19–August 19) maternity season. Partitions were established to equalize data between time periods and match our observations of pregnancy, lactation and post-lactation of females at this site (observations are based on captures for an independent study).

## Results

### *Prey identifications*

We recovered PCR products from 74% of the analysed prey fragments. Of these, 62% yielded sequences in unidirectional sequencing, most >600 bp in length. Of these, 65% were derived from prey and the rest were determined to be contaminants. We encountered a higher rate of presumed fungal and bacterial contamination than reported by Clare *et al.* (2009), and greater amplificiation of predator (bat – *M. lucifugus*) DNA (the marjority of contaminants). All sequences derrived from prey have been deposited in Dryad:doi:10.5061/dryad.8447. We successfully identified prey sequences using the BOLD reference library (http://www.barcodinglife.org) and both identification criteria. We identified 66 distinct prey species; 61 belonging to the Insecta and 5 to the Arachnida (Table 1). Most identifications were made at the species level. Higher level identifications were made only when sequences matched a reference sequence following our criteria but the reference itself lacked species level taxonomy in the BOLD database. In several cases we suspect that multiple distinct species are included in a designation but we cannot yet address these due to taxonomic ambiguity in the genera. Nearly a third (32%) of all identifications were to species in the Emphemeroptera (mayfly) genus *Caenis*. MOTU analysis estimates that the actual number of prey species is between 180 (4% threshold) and 230 (1% threshold).

### *Spatio-temporal variation in diet*

Estimates from the MOTU analysis indicate that the mean number of prey species in the diet was lower in agricultural settings than the forest roosts (Fig. 1a). The proportion of un-identifiable prey was approximately equal between the three sites: 34% in agricultural roost 1, 32% in agricultural roost 2 and 34% in the forest roost. In the forest roost, the percentage of species level identifications in the diet of *M. lucifugus* varied temporally (Fig. 1b). Dipteran (fly) species were identified in high proportions (63%) in the diet of *M. lucifugus* in early maternity season and at lower proportions in mid (10%) and late (25%) maternity season. Ephemeroptera (mayfly) were detected at lower proportions (7%) in early maternity season but dominate the diet in middle (66%) maternity season. Similar trends were observed at the other two sites.

### *Environmental indicator species*

Ephemeroptera and Diptera species were the main prey detected at all locations. Hymenoptera including the ants *Tetramorium caespitum* and *Formica ulkei*, and the sawfly *Xyela* sp. were only detected in the faeces of forest-roosting bats. Trichoptera (caddisflies) were found commonly in agricultural bats' diet but less often in forest-roosting bats.

The caddisfly *Hydropsyche betteni* occurs in small to medium sized rivers while *Ceratopsyche morosa* occurs in medium-large rivers; both were detected at agricultural roost 1. *C. morosa* has a minimal pollution tolerance but no identified species have exceptionally low tolerances (Lenat 1993, unpublished US EPS data). None of the taxa from agricultural roost 2 or the forest roost are diagnostic of river or stream habitat: *Nemotaulis hostilis, Triaenodes injustus* and *Agrypnia vestita* (agricultural roost 2), *Anabolia bimaculata*, *Triaenodes injustus* and *Triaenodes tardus* (forest roost) suggest a pond or lake

**Table 1** Species detected in the diet of *Myotis lucifugus.* Roost sample numbers represent total number of positive identifications at each maternity roost over all analysed collections (AR1 = agricultural roost 1, AR2 = agricultural roost 2, FR = forest roost). Maternity roosts were sampled from May to August

| Identifications | | | | | *Frequency | | |
|---|---|---|---|---|---|---|---|
| Class | Order | Family | Genus | Species | AR1 | AR2 | FR |
| Arachnida | Araneae | Araneidae | *Larinioides* | *Larinioides patagiatus* | | | 1 |
| | | Theridiidae | *Enoplognatha* | *Enoplognatha* sp. | 1 | | |
| | Trombidiformes | Arrenuridae | *Arrenurus* | *Arrenurus* sp. | | | 1 |
| | | Unionicolidae | *Neumania* | *Neumania* sp. | | | 1 |
| | | | *Unionicola* | *Unionicola* sp. | | | 2 |
| Insecta | Coleoptera | | | *Genus* sp. | 1 | | |
| | | Carabidae | *Amara* | *Amara* sp. | | | 1 |
| | | Chrysomelidae | | *Genus* sp. | | 1 | |
| | | Curculionidae | *Hypera* | *Hypera* sp. | | | 2 |
| | | | *Phyllobius* | *Phyllobius oblongus* | 1 | | |
| | | | *Polydrusus* | *Polydrusus sericeus* | | | 2 |
| | | Dermestidae | *Attagenus* | *Attagenus unicolor* | | 3 | |
| | | Nitidulidae | | *Genus* sp. | 1 | | |
| | | Scarabaeidae | *Amphimallon* | *Amphimallon majale* | | | 3 |
| | | | *Phyllophaga* | *Phyllophaga futilis* | | | 7 |
| | | | | *Phyllophaga* sp. ‡ | | | 4 |
| | | Scirtidae | *Cyphon* | *Cyphon laevipennis* | | | 1 |
| | Diptera | | | *Genus* sp. | 3 | 8 | 4 |
| | | Anthomyiidae | *Delia* | *Delia antiqua* | | 2 | |
| | | Calliphoridae | *Pollenia* | *Pollenia* sp. | 1 | | |
| | | Chironomidae | | *Genus* sp.‡ | 1 | | 26 |
| | | | *Chironomus* | *Chironomus decorus* | | 3 | 4 |
| | | | | *Chironomus dilutus* | 1 | 2 | |
| | | | | *Chironomus entis* | | 6 | 2 |
| | | | | *Chironomus* sp.‡ | | 5 | 1 |
| | | Drosophilidae | *Chymomyza* | *Chymomyza amoena* | 1 | | |
| | | Limoniidae | *Limonia* | *Limonia* sp. | | | 1 |
| | | Psychodidae | | *Genus* sp. | 8 | 1 | 12 |
| | | Scathophagidae | | *Genus* sp. | | | 2 |
| | | Tipulidae | | *Genus* sp. | 1 | | 4 |
| | Ephemeroptera | Caenidae | *Caenis* | *Caenis latipennis* | 2 | | |
| | | | | *Caenis youngi* | 4 | | 28 |
| | | | | *Caenis* sp. ‡ | | 27 | 35 |
| | | Ephemerellidae | *Eurylophella* | *Eurylophella temporalis* | | | 2 |
| | | Heptageniidae | *Maccaffertium* | *Maccaffertium mediopunctatum* | 6 | | |
| | | | *Stenacron* | *Stenacron interpunctatum* | 1 | | |
| | | | *Stenonema* | *Stenonema femoratum* | 1 | | 1 |
| | | Leptohyphidae | *Tricorythodes* | *Tricorythodes* sp. | 1 | | |
| | Hemiptera | Aphididae | *Euceraphis* | *Euceraphis* sp. | | 1 | |
| | | Notonectidae | *Notonecta* | *Notonecta* sp. | | | 3 |
| | Hymenoptera | Formicidae | *Formica* | *Formica ulkei* | | | 1 |
| | | | *Tetramorium* | *Tetramorium caespitum* | | | 7 |
| | | Xyelidae | *Xyela* | *Xyela* sp. | | | 1 |
| | Lepidoptera | Coleophoridae | *Blastobasis* | *Blastobasis glandulella* | | 1 | |
| | | Crambidae | *Crambus* | *Crambus agitatellus* | | | 2 |
| | | Gelechiidae | *Scrobipalpa* | *Scrobipalpa atriplicella* | 1 | | |
| | | Hepialidae | *Korscheltellus* | *Korscheltellus lupulina* | | | 6 |
| | | Noctuidae | *Hypena* | *Hypena sordidula* | | 1 | |
| | | Pterophoridae | *Hellinsia* | *Hellinsia homodactylus* | | 1 | |
| | | Sphingidae | *Deidamia* | *Deidamia inscriptum* | | | 1 |
| | | Tineidae | *Diachorisia* | *Diachorisia velatella* | | 1 | |
| | | Tortricidae | *Eucosma* | *Eucosma cataclystiana* | | 1 | |

**Table 1** (Continued)

| Identifications | | | | | *Frequency | | |
|---|---|---|---|---|---|---|---|
| Class | Order | Family | Genus | Species | AR1 | AR2 | FR |
| | | | | *Eucosma derelecta* | | | 1 |
| | | | | *Eucosma* sp. | | 1 | |
| | | | *Pandemis* | *Pandemis* sp. | | 1 | |
| | Neuroptera | Hemerobiidae | | *Genus* sp.‡ | | 2 | |
| | Plecoptera | Perlidae | *Perlesta* | *Perlesta* sp. | 1 | | |
| | Trichoptera | Dipseudopsidae | *Phylocentropus* | *Phylocentropus* sp. | | 2 | |
| | | Hydropsychidae | *Ceratopsyche* | *Ceratopsyche morosa* | 1 | | |
| | | | *Hydropsyche* | *Hydropsyche betteni* | 2 | | |
| | | | *Triaenodes* | *Triaenodes connatus* | | | 1 |
| | | | | *Triaenodes injustus* | | 12 | 1 |
| | | | | *Triaenodes tardus* | | | 1 |
| | | Limnephilidae | *Anabolia* | *Anabolia bimaculata* | | | 5 |
| | | | *Nemotaulius* | *Nemotaulius hostilis* | | 1 | |
| | | Phryganeidae | *Agrypnia* | *Agrypnia vestita* | | 1 | |

*Detection numbers represent conservative estimates and likely underestimate actual presence. Detections are assumed to be independent – see discussion for limitations of abundance data.
‡>1 species suspected.

habitat. Most of the other species identified at these two sites can occur in river, stream, pond or lake habitats in the study region.

## Discussion

In this study we use the molecular methods of Clare *et al.* (2009) to identify the prey of the generalist insectivore predator *M. lucifugus* in Southwestern Ontario and to identify spatial–temporal patterns of predation. Our analysis recovered the identities of 66 distinct prey species in the diet of *M. lucifugus* and establishes that these bats rely heavily on mass-emerging aquatic insect species such as the mayfly genus *Caenis*, though the reliance on prey groups changed seasonally. Bats roosting in a forested setting consumed a wider variety of prey species than those found in agricultural locations. Species level identifications of prey make it possible to predict the type and health of the aquatic system being used by each group of bats.

### *Molecular methods of species identification*

We analysed 96 insect fragments from each collection made at each maternity roost. The choice of $n = 96$ fragments analysed (exploiting 96-well DNA extraction plates) acknowledges that many bats will have contributed to the pooled sample collected under each roost bi-weekly, thus the strategy of Clare *et al.* (2009) (16 fragments/bat) would not be sufficient. We encountered lower amplification and sequencing success and more fungal and bacterial contamination than Clare *et al.* (2009). Most samples sat on collection sheets for up to a week before preservation making our collections noninvasive but reducing the efficiency of our analysis. Fungal and bat DNA was frequently amplified, a problem not encountered in Clare *et al.* (2009). The primers used here have greater affinity for *Myotis lucifugus* DNA (this study) than *Lasiurus borealis* (Clare *et al.* 2009) which is consistently difficult to amplify at the COI region (E. Clare, unpublished). Future studies would benefit from the use of blocking primers (Deagle *et al.* 2009) to reduce nontarget DNA amplification and sequencing. We also encountered more sequences which could not be fully identified to species than Clare *et al.* (2009). *M. lucifugus* consumes insects underrepresented in public DNA databases while *L. borealis* (Clare *et al.* 2009) consumes primarily Lepidoptera (moths and butterflies), the subject of a major campaign within DNA barcoding (http://www.lepbarcodinge.org) and heavily populating public repositories like BOLD (http://www.barcodinglife.org) which act as a reference database for our identifications.

In the absence of a tight sequence match (e.g. <2% divergence), assigning a sequence to a higher taxonomic level based on sequence similarity alone is error prone. Even with more than 600 bp amplified here, mitochondrial DNA such as COI lacks sufficient phylogenetic signal to support generic, family or ordinal-level taxonomic assignments unless the target fauna has been comprehensively barcoded, and even then assignment above genus is difficult (Wilson 2010). This problem increases when smaller regions are targeted (e.g. only an ~130 bp overlap exists between

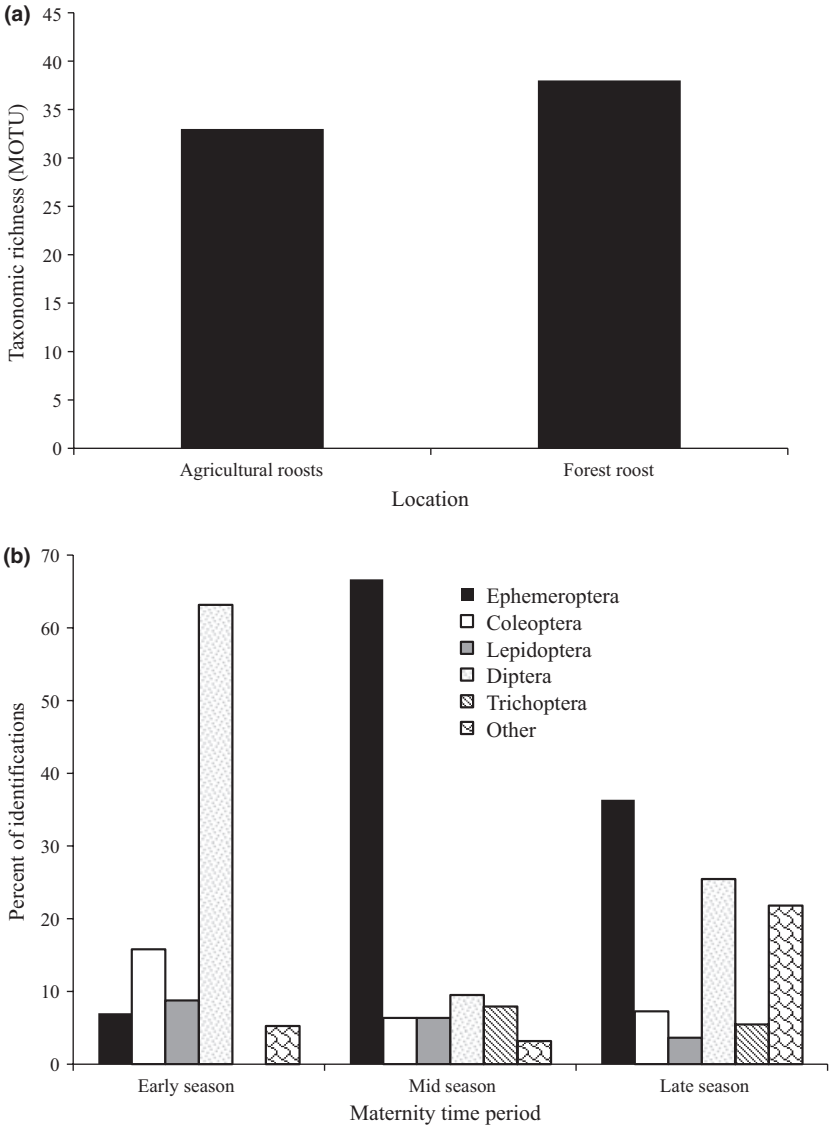


**Fig. 1** Spatial–temporal heterogeneity in dietary richness. (a) Maternity roosts in different habitats showed different dietary richness with the largest number of species consumed by forest roosting bats. (b) Shifts in species consumption occurred during the three temporal intervals of our analysis at the forest roost. Early in the season a diverse group of Dipteran species were consumed while Ephemeroptera dominated in mid season.

the region amplified by the primers described by Zeale *et al.* (2011) and the standard region amplified by DNA barcoding).

Higher level taxonomic assignments may be correct in some cases but, in our experience, unidentified sequences will frequently show significant similarity to reference sequences in divergent groups. In this study we encountered numerous examples of sequences that lacked a tight sequence match to any known species, but showed similarity values >85% to species in several different insect orders, very likely reflecting substitution saturation. While this does not negate the use of mini-barcodes (Hajibabaei *et al.* 2006a) for species level identifications when a comprehensive barcode library is available, higher taxonomic assignments are risky, particularly so when sequences are short or of poor quality.

To overcome the problem of unidentified prey sequences, we combined taxonomic identifications with the MOTU approach to estimate prey species diversity in the samples from our sites. The advantage of MOTU analysis is that it can be applied to unidentified sequences (those for which reference species do not exist but nevertheless represent distinct prey) in any analysis. For example, the diversity of prey in a more natural area may include rare species which are less likely to populate reference databases. These would be un-identifiable but an important component of dietary richness. MOTU-type analyses allow us to estimate this un-quantified diversity and will be particularly useful when addressing niche size and overlap issues in fauna from areas lacking any reference database. jMOTU requires a specific user-defined threshold (or set of thresholds). Ultimately, a complete reference database

is required to complete these analyses, however the choices of 1–4% cutoff values used here are biologically realistic (e.g. intraspecific divergence up to 3.7% in Hesperiidae, 4.6% in Sphingidae and 2.2% in Saturniidae, Hajibabaei *et al.* 2006b). The relationship between threshold and MOTU analysis is correlated but reaches a plateau within the 'barcode gap' where within-taxon diversity is covered, but taxa have not been collapsed. Thus, at least at biologically relevant levels, determining an exact threshold should not be necessary to infer a trend. The combined approach of forensic taxonomy with bioinformatics techniques used here has provided the highest taxonomic resolution for the diet of *M. lucifugus* to date and strongly supports the use of molecular methods for dietary analysis in insectivorous groups.

We treated each identified insect fragment as an independent incidence of consumption. This relies on the assumption that we have not resampled the same insect, either via two guano samples from the same bat or two insect samples from the same piece of guano. Since these roosts may contain thousands of bats, the chance that we subsampled guano from the same individual is remote, particularly since we observed individuals moving around the roost nightly so that the same bats were not consistently located over our collection sheets. Additionally, all samples were placed in a collection bag (volume: up to 1 L of guano each week) and from these a sample of guano pieces was removed for dissection, minimizing the chance of resampling a single individual's guano. We attempted to sample insect fragments from a large number of guano pellets, and since each pellet may contain prey of many species (Clare *et al.* 2009), we consider the chance of resampling an individual insect as low. However, we caution that these results should be treated as semi-quantitative only.

### *Spatial heterogeneity in diet – habitat influences*

The variability of the diet of bats roosting in the $\sim$1 km$^2$ forested patch of the forest roost was higher than that of colonies in agricultural habitats which may provide less prey variety due to habitat disturbance (Blair & Launer 1997). Walsh & Harris (1996) suggested that prey diversity along woodland edges make them a preferred habitat of Vespertilionid bats, particularly when they are near water bodies, and this accurately describes the forest roost habitat. More natural habitats likely include more rare species which are less likely to appear in public repositories. These would result in un-identifiable MOTU in our analysis. Interestingly, the proportion of un-identifiable prey (difference between MOTU and actual ID) was not skewed towards the forest roost. Though we cannot generalize beyond these three colony locations, it would be interesting to conduct a more widespread analysis of the influence of natural habitat, conservation and reclaimed habitat, and mixed and monoculture farming habitat on insect diversity and bat diet to clarify this issue. Agricultural habitats like Clinton (agricultural roost 1) and Norfolk County (agricultural roost 2) may provide less variety in prey species due to urbanization and habitat disturbance caused by farming (Blair & Launer 1997). Just as some insect species provide an accurate indicator of the health of the aquatic system, some insect species may act as strong indicators of other habitat types. Formicids are important components of temperate forest ecosystems (Lynch *et al.* 1988) and were detected only in faeces from forest roosting bats – suggesting even small patches may increase dietary richness provided they have the correct ecosystem components.

### *Temporal heterogeneity in diet – seasonal influences*

The prey species identified in the diet of *M. lucifugus* showed temporal variation between early, middle and late maternity season. Diptera and Ephemeroptera, specifically Chironomidae and the mass emerging mayfly *Caenis* sp., were consumed in large quantities throughout the maternity season and similar observations were made in previous morphological analyses (Kunz 1974; Belwood & Fenton 1976). These insects are small in size, but likely represent an abundant prey source (Kunz 1974; Belwood & Fenton 1976) which persists for many days – and targeting these species may be an advantageous strategy for colonially roosting insectivores. The diets of lactating *M. lucifugus* are more variable than adult males and this is thought to be due to increased energy requirements of reproduction (Belwood & Fenton 1976). We recorded a substantial switch from a primarily mixed dipteran diet to a strong reliance on mass-emerging Ephemeroptera at the transition from early to middle maternity season. This coincides with our observations of parturition – potentially indicating an energetically driven dietary switch, though the cause–effect relationship is not clear and requires considerably more intensive study.

### *Specialists vs. generalists*

In conservation planning specifically, and organismal biology generally, species are often classified as 'specialists' or 'generalists' – particularly with respect to their feeding niche. If we consider the dietary profile of *M. lucifugus* (66 prey species at 3 locations, from hundreds of bats) with that of *L. borealis* (127 prey species from 56 bats at only one location) (Clare *et al.* 2009) the dietary

richness of *L. borealis* is larger. From this perspective, *L. borealis* could be classified as a 'generalist' while *M. lucifugus* would be the 'specialist'. However, if we consider their diets phylogenetically, *L. borealis* diet is almost entirely composed of Lepidopteran species (Clare *et al.* 2009) while *M. lucifugus* consumed prey from a more even distribution of prey groups. From this perspective, *M. lucifugus* is the generalist and *L. borealis* the specialist. Clearly the comparison should be considered carefully; however, it is apparent that ecological classification depends on perspective (see review by Devictor *et al.* 2010).

### *The influence of water quality on the food chain*

Our data indicate that bats using agricultural roost 1 fed over rivers or streams of fair water quality. There were no species detected at any roost which have an exceptionally low pollution tolerance value, thus no habitat can be classified as excellent. In addition, the more limited dietary fauna estimated for bats at agricultural roosts suggest a lower diversity of prey and hence nonpristine aquatic source habitat. Mayfly taxa at agricultural roost 1 (*Caenis* sp., *Stenacron interpunctatum*, *Stenonema femoratum*, *Maccaffertium mediopunctatum*) are diagnostic of moving water habitats in the mid-Atlantic part of their range but can be found in lakes, small streams and medium sized rivers in our northern areas. Unlike the other sites, there were no dietary species detected at agricultural roost 1 that specifically suggest pond or lake environments. There are several local rivers that could support these dietary species and we predict that these are much more likely foraging habitats.

Many prey associated with bats from agricultural roost 2 and the forest roost are diagnostic of ponds or lakes while others can occur in ponds, lakes, rivers and streams. From this we conclude that, unlike bats of agricultural roost 1, these colonies forage over ponds or lakes rather than flowing water. Direct observation of bats using the forest roost suggests they feed over Lake St. George, <300 m from the roost, supporting this conclusion. In more northern areas, such as our study site, habitat specificity decreases and indicator species are not as diagnostic as they are in more southerly ranges however we were able to draw conclusions about habitat; the insects identified indicate that these bats are not using temporary drainage ditches, artificial canals, industrial outflows, etc. but bats in similar areas are relying on different water sources, ponds or lakes vs. rivers or streams, either directly as foraging habitat or indirectly as the source of their insects. This system provides an excellent model to examine the influence of habitat health up a food chain.

## Conclusions

In our analysis we positively confirmed the presence of 66 different species of prey in the guano of *Myotis lucifugus* and, using these data, we detected significant spatial and temporal patterns in diet. Individuals roosting in agricultural settings had lower dietary richness than the roost located in a forested environment. We detected temporal fluctuations in diet between early, middle and late maternity season suggesting a strong reliance on only a few species of mass emerging prey. The species detected suggested that bats at agricultural roost 1 forage at local river sites while colonies at agricultural roost 2 and the forest roost feed on or near still water, such as pond or lake habitats, rather than a flowing stream or river. All water sources are of fair to good quality, though none of the species detected indicate pristine habitat. Our study outlines a model system to study the relationship between abiotic and biotic factors – from interactions between habitat and water quality through this simple food chain to the top predator.

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