Resource Partitioning by Insectivorous Bats in Jamaica

Matthew A. Emrich\textsuperscript{1}, Elizabeth L. Clare\textsuperscript{2}, William O.C. Symondson\textsuperscript{3}, Susan E. Koenig\textsuperscript{4}, and M. Brock Fenton\textsuperscript{1}

\textsuperscript{1}Department of Biology, Western University, London, Ontario N6A 5B7, Canada,

\textsuperscript{2}School of Biological and Chemical Sciences, Queen Mary University of London, Mile End Road, London E1 4NS, UK.

\textsuperscript{3}Cardiff School of Biosciences, Cardiff University, Sir Martin Evans Building, Museum Avenue, Cardiff, CF10 3AX, UK.

\textsuperscript{4}Windsor Research Centre, Sherwood Content P.O., Trelawny, Jamaica.

Corresponding Author: E.L. Clare. School of Biological and Chemical Sciences, Queen Mary University of London, Mile End Road, London E1 4NS, UK, e.clare@qmul.ac.uk, Fax: +44 (0)20 7882 7732

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Abstract

In this investigation we use variation in wing morphology, echolocation behaviour, patterns of habitat use and molecular diet analysis to demonstrate that six species of sympatric insectivorous bats in Jamaica showed significant differences that could explain resource partitioning among the species. High intensity echolocating species that used shorter, broadband signals and had shorter, broader wings (*Pteronotus macleayii, Pteronotus quadridens, Mormoops blainvillii*) foraged most in edge habitats but differed in timing of peak activity there. *P. macleayii* and *M. blainvillii* differed in diet but low sample size precluded diet analysis for *P. quadridens*. High intensity echolocating species that used longer, more narrowband signals and had longer, narrower wings (*Molossus molossus, Tadarida brasiliensis*), foraged most in open areas, and differed in diet from the other species. Two disparate species were most active in clutter (dense vegetation). *Pteronotus parnellii* used high duty-cycle echolocation apparently specialized for detecting fluttering targets in clutter. *Macrotus waterhousii* used low intensity, broadband echolocation calls and presumably uses prey-generated sounds when foraging. – These two species also differed in diet. Our data show that differences in morphology and echolocation behaviour coincide with differences in habitat use and diet, resulting in minimal overlap in resource use among species.
Introduction

Sympatric animal species, especially those generally similar in morphology, are thought to coexist by partitioning available limiting resources (Ricklefs 2007). Schoener (1974) outlined other potential axes for partitioning and noted that partitioning of space was common among some sympatric species, but habitat use, diet, and time could also influence partitioning. These factors may, by themselves or in combination allow resource partitioning. But what happens when or if resources are abundant? The abundance and diversity of nocturnal insects may underlie the origin and diversification of bats (e.g., Fenton et al. 1995) but it is not clear if, when, and how contemporary communities actively partition resources. In particular, does partitioning by habitat translate into partitioning by diet? For bats, perhaps particularly insectivorous species, there is little evidence of direct competition for food resources, although differences in habitat use may generate variation in diet.

For insectivorous bats, wing morphology and echolocation can be two major factors associated with niche partitioning that may interact with prey consumption. Insectivorous bats with short rounded wings appear adapted to forage in edge situations, while those with longer, more pointed wings forage in more open habitat (Aldridge & Rautenbach 1987; Norberg & Rayner 1987). For operation in clutter, areas where many echoes rebound from vegetation, wing shape may be a less important determinant of habitat use than echolocation and ability to detect prey (Aldridge & Rautenbach 1987). Bats use two main approaches to echolocation. Low duty-cycle echolocators separate outgoing pulse from returning echoes in time while high duty-cycle echolocators separate them in frequency (Fenton et al. 2012). Some bats that use low duty-cycle echolocation produce narrowband search phase echolocation calls and hunt in the open (Aldridge & Rautenbach 1987; Schnitzler et al. 2003). Other low duty cycle species use calls dominated by...
broadband frequency modulated sweeps, and tend to forage in edge situations (Aldridge &
Rautenbach 1987; Schnitzler et al. 2003). Bats using high duty-cycle echolocation produce
echo location calls dominated by a constant frequency and are well suited to detecting fluttering
targets in clutter (Fenton et al. 2012). Some low duty-cycle bats that produce low intensity,
multiharmonic broadband signals hunt in cluttered settings, detect prey by sounds the prey
produce (Bell 1985) and more often hunt in cluttered situations. In general, for low duty-cycle
echolocators, longer search phase signals that are narrower in bandwidth give more effective
operational range than shorter, broadband signals (Simmons and Stein 1980).

The question of whether habitat partitioning translates into dietary partitioning is
particularly relevant for sympatric species in communities of insectivorous bats in tropical and
subtropical communities where there is greater potential for competition (Findley 1993).
Differences in wing morphology (Aldridge & Rautenbach 1987; Norberg & Rayner 1987) and/or
bite force (Santana & Dumont 2009), echolocation call design (Aldridge & Rautenbach 1987;
Norberg & Rayner 1987), habitat use (Kunz 1973; Hickey, Acharya & Pennington 1996), and
diet (Santana, Dumont & Davis 2010) may all result in partitioning and co-existence without
overt competition for resources (e.g., Santana & Dumont 2009). Among insectivorous bats,
Mancina et al. (2012) proposed that partitioning in a community of mormoopid bats (*Pteronotus
parnellii, P. macleayii, Mormoops blainvillii*) involved a combination of morphology,
echo location behaviour, and time.

We use modern and traditional approaches to assess the potential for resource partitioning
among six sympatric species of insectivorous bats that roost and forage in the same area.
Specifically, we used flight path reconstructions and molecular analysis of diet along with
traditional measures of wing morphology and echolocation calls. The six species we studied
include *Pteronotus parnelli* (Fig. 1) (Gray 1843), *Pteronotus macleayii* (Gray 1843), *Mormoops blainvillii* Leach 1821 (Mormoopidae), *Macrotus waterhousii* Gray 1843 (Phyllostomidae), *Tadarida brasiliensis* (I. Geoffroy, 1824) and *Molossus molossus* (Pallas 1766) (Molossidae). A seventh species, *Pteronotus quadridens* (Gundlach 1840), was included in analyses of flight behaviour, wing morphology and echolocation call characteristics but inadequate sample sizes precluded analysis of its diet. We tested the prediction that species-specific characteristics (e.g., wing morphology and echolocation behaviour), traditionally used to infer mechanisms of resource partitioning are reflected by quantifiable differences in habitat use and diet.

**Methods**

We worked in Jamaica near Windsor Cave (18°21′N, 77°38′W, elevation 100-500 m), a large daytime roost for the above-referenced species, except for *M. molossus* which roosts in houses in the study area. An additional insectivore, *Chilonatalus micropus*, roosts in the cave but was rarely encountered in our work. Land use is a mix of disturbed closed-canopy evergreen broadleaf forest, agriculture fields of sugar cane, pasture, and coffee, and riparian forest along the banks of the Martha Brae river. According to Genoways et al. (2005), six additional insectivorous species occur in Jamaica, but they were not encountered in our work. We captured bats in mist nets and harp traps from 4 December 2010 – 2 April 2011, 13 July - 6 August 2011, and 12 May - 9 June 2012. Following Aldridge and Rautenbach (1987), we recorded mass, species, sex, reproductive status, age, forearm length (fl), length of wing hand (lwh), length of arm wing (law) and body width (bw). We held bats individually in cotton bags to collect fecal samples from them. Fecal samples were frozen within 12 h of collection and bats were released within 60 min of capture. We photographed the right wing and tail membrane.
against graph paper with a grid of 5 mm for individuals of each species. From these photographs we calculated total surface area (S) and surface area of the hand wing (Shw) and arm wing (Saw), wingspan (b), aspect ratio (AR), wing loading (Wl), tip length ratio (Tl), tip area ratio (Ts) and tip shape index (I) (Norberg & Rayner 1987). For species captured all year, we divided fecal samples based on collections made during dry (December 2010 to March 2011) and wet (July to August 2011) seasons.

To assess habitat use, we deployed two four-microphone arrays (see Supplemental File) to acoustically monitor 9 sites (minimum 5 nights each), representing cluttered, open, and edge habitats (Schnitzler et al. 2003). These sites were within a circle with a radius of 750 m. The first site (Site 1) for acoustic monitoring was the front yard of a home. Site 2 was a cliff face that overlooked tree canopy. Site 3 was an area that had been cleared for cultivation but has since been abandoned, it was composed mostly of ferns. Site 4 was a section of river located in a cluttered habitat. Site 5 was a section of river located in an open habitat. Site 6 was the boundary of a cluttered forest and an open pasture. Site 7 was a small patch of forest surrounded by roads and open habitats. Site 8 was a sloped hillside along a forest trail. Site 9 was a forested plateau located between the peaks of two hills. We recorded echolocation activity continuously from 50 minutes before to 790 minutes after sunset using Avisoft Recorder USG software (Avisoft Bioacoustics, Berlin, Germany) with a 250 kHz sampling frequency, and 8 bit format. We used callViewer18 (Skowronski & Fenton 2008) to assign echolocation calls to species. We used an activity index (AI) (Miller 2001) to assess levels of bat activity by habitat. AI is based on the number of one minute long files in which a species was detected, modified for relative habitat use (species AI on a given night / total AI for the species). We used principal components analysis (PCA) on relative AI to reduce dimensionality of habitat use and SaTScan (v.9.1.1;
SaTScan, Boston, USA) to compare activity levels between sites to identify periods of high and low activity. SaTScan is designed to discover statistical significances of disease outbreaks across space and time. The same principals used by the software to analyze the occurrence of diseases can also be applied to determine peak activity (A. Adams, unpublished). This approach allowed us to examine levels of activity across all sites and determining the probability that one peak in activity was greater than peaks in other locations.

To examine flight behaviour, we generated estimated flight paths with MatLab Moonshine (Lasse Jakobsen, University of Ulm). Moonshine analyzes sequences of 3 - 30 calls and calculates the bat’s position in space and time (Brinklov et al. 2011). Total flight path speeds were calculated by taking the total distance traveled within a flight path and dividing it by the time.

To determine diet, we analyzed fecal samples from 8 *M. blainvillii* and 8 *M. molossus* (collected in the wet season) and 16 each of *P. parnellii*, *T. brasiliensis*, *P. macleayii*, *M. waterhousii* (8 in each season) (n = 80). We analyzed diet using the Roche 454 next generation sequencing protocols modified from Bohmann et al. (2011) (Supplemental file) and calculated the number of molecular operational taxonomic units (MOTU) consumed by each species in each pooled sample (Bio-informatics in supplemental file).

**Statistical Analyses**

To determine morphological partitioning, we used independent sample Kruskal-Wallis tests to compare morphological values among species with non-normal distributions. To determine behavioural partitioning associated with flight speeds, we ran Conover-Inman test for all possible pairwise comparisons of mean flight speeds between species which determined if
they were statistically the same. To assess partitioning by diet, we used the Sørensen Similarity Index (McCune, Grace & Urban 2002) and Minimum Hamming Distances (Hamming 1950) to compare diets among species and between seasons.

Results

We found significant differences in wing morphology (Table 1) among species. *Tadarida brasiliensis* and *M. molossus* have longer, narrower, more pointed wings, with high wing loadings, high aspect ratios and fast flight speeds. Therefore we expected them to forage most often in open habitats. *Pteronotus macleayii*, *P. quadridens* and *M. blainvillii* have short and rounded wings, low wing loadings, low aspect ratios and intermediate flight speeds and should be most active in edge habitats. *Pteronotus parnellii* has a combination of broad wings, rounded wingtips, low wing loading, low aspect ratios and slow flight speeds and should be most active in clutter. *Macrotus waterhousii* should be most active in clutter because of its combination of details of wing and echolocation call and hunting behaviour. *P. parnellii* flew significantly more slowly than *T. brasiliensis* and *M. blainvillii* while *P. macleayii* flew at intermediate speeds that did not differ significantly from those of any other species.

Interpretation of the search phase echolocation calls (Table 2) of these species generally supports morphological categorizations; *P. macleayii*, *P. quadridens* and *M. blainvillii* use broadband calls which provide details about prey but at shorter range resolution suggesting adaptation for foraging in edge situations (Table 2). *Tadarida brasiliensis* and *M. molossus* use longer, narrowband signals well suited for foraging in the open. The high duty cycle echolocation behaviour of *P. parnellii* suggests that it is well suited for hunting fluttering targets (flying insects) in clutter (Lazure & Fenton 2011; Fenton, Faure & Ratcliffe 2012). *Macrotus*
waterhousii also should be most active in clutter because of low intensity echolocation calls, and
detection of prey through sounds they generate (Bell 1985).

Patterns of habitat use (Fig. 12) matched predictions arising from wing morphology and
echolocation call design (above). We observed that T. brasiliensis and M. molossus were most
active in open areas while M. blainvillii, P. quadridens and P. macleayii use edge habitat (Fig.
21). Wing structure and its high duty-cycle echolocation characteristic suggested that P. parnellii
would be most active in clutter habitats and this was confirmed by our observations. Due to their
low intensity echolocation calls, M. waterhousii was not detected in our acoustic survey.

Where two species used the same habitat, their activity was temporally displaced, e.g.,
activity of M. blainvillii peaked later than that of P. macleayi in edge habitats (Fig. 23). Sites 3
and 7 were not included in Fig. 23 due to a high level of spatial partitioning (one dominant
species using the site).

We recovered 119 101 raw sequencing reads. After bioinformatics processing, we
reduced these to 53 330 unique haplotypes. Collectively the species we studied consumed These
were clustered into 616 species (MOTU) from a wide variety of insect orders (Fig. 43) (see
Supplemental file). Overall, we found low levels of dietary overlap among species (Table 3),
including those that foraged in edge (P. macleayi, M. blainvillii), open (M. molossus and T.
brasiliensis) and clutter (P. parnellii and M. waterhousii; latter is presumed). We also found low
overlap between dry and wet seasons in T. brasiliensis, M. waterhousii, P. macleayi and P.
parnellii. Of the total 616 insect species consumed, only 88 were found in both wet and dry
seasons. Molossus molossus and M. blainvillii were not compared between seasons because of
small sample sizes.
Discussion

There are multiple potential mechanisms of resource partitioning and they are not necessarily independent. For example, partitioning by habitat may lead to apparent dietary partitioning though the mechanism is habitat choice rather than competition. Differentiating between present competitive interactions, secondary effects and the residual effects of past competition are nearly impossible without controlled removal experiments. Understanding the mechanism and causes of partitioning may be particularly difficult on islands where the fauna may be species poor in some aspects, but composed of a mixture of species which did not evolved in sympatry but colonized in different dispersal waves. In these cases, current ecosystem dynamics may represent a mix of occupation of empty niches, historical competitive interactions, exaptations and behaviours originating from historical contingencies rather than current interactions (adaptive in the ecosystem of origin but no longer useful).

Our results are similar to those from other studies (e.g. Aldridge & Rautenbach 1987; Mancina et al. 2012) that reported how differences in wing morphology and echolocation call design could result in resource partitioning. We found that differences in wing morphology and echolocation behaviour coincided with differences in details of flight behaviour, habitat use, and diet. The net effect is resource partitioning through a combination of habitat, temporal shifts in activity and diet. Importantly, our findings extend previous studies by considering multiple (rather than two) dimensions of partitioning within a bat community (Kunz 1973; Razgour, Korine & Saltz 2011, Nicholls & Racey 2006), and demonstrate partitioning even in heavily used edge habitats.——Previous evidence that bats partition time in their patterns of habitat use has been
limited to areas where water is limiting (Razgour et al. 2011; Adams & Thibault 2006). A new
application of SaTScan allowed us to detect previously overlooked temporal partitioning, e.g. at
Site 6, one of the most active sites used by the most species, each species had a unique set of
high and low activity times with minimal overlap among them (Fig. 23).

DNA barcoding provided greater precision of analysis of diet than previous studies of
sympatric bats (Fukui, Okazaki & Maea 2009; Hickey et al. 1996). Our results clearly indicate
that species ate different insects, and that there was little overlap in their diets between wet and
dry seasons likely due to differences in insect availability through changing life cycle and
activity patterns. These differences coincide with variation in morphology and echolocation
behaviour. Our data suggest that *P. parnellii* was the dominant consumer of moths (Table 2).

*Pteronotus parnellii* and other species in this complex (Clare et al. 2013) are high-duty cycle
echolocators (like old world members of the families Rhinolophidae and Hipposideridae). High
duty cycle echolocation provides better detection of fluttering targets, particularly in cluttered
situations (Lazure & Fenton 2011; Fenton et al. 2012). The analysis in Fig. 34 suggests that *P.
parnellii* ate more moths than any of the other species in this community. But, the values at
nodes represent the number of species-level BLAST assignments for a given taxon. A high rate
of false positive assignments of COI at higher taxonomic levels such as tribe and family (Wilson
et al. 2011) has been observed but order level assignments may actually be relatively robust
under certain informatic protocols (Clare unpublished data). While this means that any one
assignment should be treated conservatively, however, higher node assignments in our analysis
likely translate into higher support for a given node as more independent assignments to the
same taxa decrease the likelihood of a false positive. This analysis suggests the importance of
Lepidoptera in the diet of *P. parnellii* but identification to the species-level requires a DNA
library of local species, something not yet available for Jamaica. *Pteronotus macleayii* consumed
the highest diversity of prey with assignments at the widest variety of nodes (Fig. 34) even
though our sample for this species was limited.

According to the competitive exclusion principle, two species coexist in a stable
environment only if they occupy niches that differ in some measure (Hardin 1960; Chesson
2000). We demonstrate how differences in morphology and echolocation behaviour coincide
with differences in habitat use and diet. Although our data suggest partitioning by diet, we
did not perform exclusion experiments and thus have no evidence to suggest that competition
is the cause. Even during the dry season when the diversity of insects was lower (216 species
versus 312 in the wet season) the diets of the bats showed minimal overlap. Prey availability may
not have been limiting for the bats we studied but could have lead to differentiation of niches in
the past even though it is not currently apparent (see also Andrianaivoarivelot et al 2006; Bell
1980; Fukui et al 2009).

Morphometric comparisons of faunas of insectivorous bats typically reveal a cluster of
similar species and a few that are distinctly different (e.g., Fenton 1972; Aldridge & Rautenbach
1987; Findley 1993). In other words whether there are 5 or >30 morphometrically similar taxa,
the distance to the nearest neighbour in a plot changes little, but the distance to farthest
neighbours is greater. In one example 14 sympatric species of bats ate mainly beetles and moths
(Fenton et al 1998). These bats differed in morphology and echolocation behaviour as reported
by Aldridge & Rautenbach (1987), but lack of details about the insects they consumed meant no
support for resource partitioning. Our data suggest that analyses of communities of sympatric
species of insectivorous bats will show, often minor, differences in morphology and other
features that collectively result in partitioning. We have demonstrated how differences in wing
morbology and echolocation calls and behaviour correlate with differences in habitat use and
diet.

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of Zoology, 270, 466–472.

Aldridge H, Rautenbach I (1987) Morphology, echolocation and resource partitioning in


**Data Accessibility:** Our data will be made accessible on Dryad for publication. (address to be added) Data is available on Dryad doi:10.5061/dryad.gm354

**Author’s contributions:** MAE designed and conducted field research. ELC conducted the molecular analysis. MBF supervised the project. SEK supervised field research. WOCS contributed to molecular protocols. All authors contributed to manuscript production.

**Figures legends:**

**Figure 1:** Flying *Pteronotus parrillii*
Figure 21: PCA of habitat preference of 4 insectivorous bats in Jamaica. Species are denoted by the first letters of their genus and species name. Species falling to the right of the PC1 axis are found in cluttered environments and species to the left are in open environments. Species found to the top of the PC2 axis are found in edge environments.

Figure 23: Temporal activity patterns of species throughout the night at their most preferred sites. Periods represented by the green checkered pattern are sites that have average activity level compared to activity at all other sites. Red, green, or blue represent periods of high, typical or low (respectively) activity compared to activity at all other sites and times.

Figure 34: A schematic hypothesis of the distribution of prey species consumed by bats in this ensemble. In the absence of a reference database, identifications have been made by BLAST score and are limited to hypothesis at the order level (see supplemental bioinformatics section). Values at nodes or tips represent the number of MOTU assigned to the node. The proportion of MOTU assigned by BLAST to a given taxonomic node for each predator is given by the pie chart.
Table 1. Morphological measurements and flight speeds of 7 insectivorous bat species in the Windsor region, Jamaica. Interspecific differences are represented by letters following values based on a Kruskal-Wallis test with a Conover-Iman pairwise comparison. Species sharing letters are statistically the same. Numbers in brackets next to flight speeds is the sample size. Habitat association was determined by comparing call features to work done Aldridge and Rautenbach (1987).

<table>
<thead>
<tr>
<th>Species</th>
<th>N</th>
<th>Mass (g)</th>
<th>Forearm Length (mm)</th>
<th>Aspect Ratio</th>
<th>Wing Loading (N/m²)</th>
<th>Tip Shape Index</th>
<th>Average Flight Speed (m/s)</th>
<th>Predicted Habitat</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Pteronotus parnellii</em></td>
<td>25</td>
<td>13.9±1.2 C</td>
<td>52.88±0.76 A</td>
<td>6.6±0.4 B</td>
<td>7.5±0.8 D</td>
<td>1.2±0.2 A</td>
<td>6.9±1.5 (17) B</td>
<td>Cluttered</td>
</tr>
<tr>
<td><em>Pteronotus quadridens</em></td>
<td>7</td>
<td>6.8±0.3 F</td>
<td>38.29±0.76 D</td>
<td>6.6±0.6 B</td>
<td>6.3±0.5 E</td>
<td>0.9±0.2 B</td>
<td>7.6±1.5 (14) AB</td>
<td>Edge</td>
</tr>
<tr>
<td><em>Pteronotus macleayii</em></td>
<td>9</td>
<td>7.1±0.5 F</td>
<td>43.04±0.79 B</td>
<td>7.1±0.7 B</td>
<td>5.9±0.9 E</td>
<td>1.2±0.2 A</td>
<td>7.5±1.5 (18) AB</td>
<td>Edge</td>
</tr>
<tr>
<td><em>Mormoops blainvillii</em></td>
<td>18</td>
<td>9.4±0.9 E</td>
<td>45.92±1.24 B</td>
<td>6.2±0.2 C</td>
<td>5.5±0.6 E</td>
<td>0.8±0.1 B</td>
<td>9.0±2.0 (11) A</td>
<td>Edge</td>
</tr>
<tr>
<td><em>Molossus molossus</em></td>
<td>21</td>
<td>18.7±1.2 B</td>
<td>38.17±0.75 D</td>
<td>8.2±0.5 A</td>
<td>17.3±1.7 A</td>
<td>0.6±0.1 C</td>
<td>-</td>
<td>Open</td>
</tr>
<tr>
<td><em>Tadarida brasiliensis</em></td>
<td>16</td>
<td>10.8±1.1 D</td>
<td>39.86±0.72 C</td>
<td>8.7±0.6 A</td>
<td>11.2±1.4 B</td>
<td>0.8±0.1 B</td>
<td>9.4±1.7 (8) A</td>
<td>Open</td>
</tr>
<tr>
<td><em>Macrotus waterhousii</em></td>
<td>20</td>
<td>20.8±1.7 A</td>
<td>53.19±1.13 A</td>
<td>5.8±0.4 D</td>
<td>8.8±0.8 C</td>
<td>1.4±0.4 6</td>
<td>-</td>
<td>Cluttered</td>
</tr>
</tbody>
</table>
Table 2. Call parameters of 7 Jamaican insectivorous bats based on call analysis of free flying and ziplined bats. Habitat association was determined by comparing call features to work done by Fenton (1990).

<table>
<thead>
<tr>
<th>Species</th>
<th>n</th>
<th>Duration (ms)</th>
<th>Fmax (kHz)</th>
<th>Fmin (kHz)</th>
<th>Bandwidth (kHz)</th>
<th>Duty Cycle</th>
<th>Intensity</th>
<th>Habitat Preference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Pteronotus parnellii</em></td>
<td>10</td>
<td>29.03±4.42</td>
<td>61.18±1.13</td>
<td>49.12±2.81</td>
<td>12.06±3.14</td>
<td>High</td>
<td>High</td>
<td>Cluttered</td>
</tr>
<tr>
<td><em>Pteronotus quadridens</em></td>
<td>10</td>
<td>4.49±0.792</td>
<td>80.03±1.43</td>
<td>60.84±1.51</td>
<td>19.19±2.26</td>
<td>Low</td>
<td>High</td>
<td>Edge</td>
</tr>
<tr>
<td><em>Pteronotus macleayii</em></td>
<td>10</td>
<td>4.80±1.21</td>
<td>70.65±1.81</td>
<td>54.69±1.15</td>
<td>15.97±1.99</td>
<td>Low</td>
<td>High</td>
<td>Edge</td>
</tr>
<tr>
<td><em>Mormoops blainvillii</em></td>
<td>10</td>
<td>2.95±1.13</td>
<td>66.65±1.87</td>
<td>44.09±3.64</td>
<td>22.56±4.08</td>
<td>Low</td>
<td>High</td>
<td>Edge</td>
</tr>
<tr>
<td><em>Molossus molossus</em></td>
<td>10</td>
<td>6.48±1.80</td>
<td>40.97±3.46</td>
<td>33.54±4.43</td>
<td>7.42±1.62</td>
<td>Low</td>
<td>High</td>
<td>Open</td>
</tr>
<tr>
<td><em>Tadarida brasiliensis</em></td>
<td>10</td>
<td>9.49±1.49</td>
<td>40.38±3.27</td>
<td>32.71±2.90</td>
<td>7.67±1.87</td>
<td>Low</td>
<td>High</td>
<td>Open</td>
</tr>
<tr>
<td><em>Macrotus waterhousii</em></td>
<td>10</td>
<td>1.91±0.71</td>
<td>73.65±6.62</td>
<td>46.19±2.68</td>
<td>27.46±7.12</td>
<td>Low</td>
<td>Low</td>
<td>Cluttered</td>
</tr>
</tbody>
</table>

* Call parameter were analyzed for ziplined individuals.
Table 3. Estimates of dietary overlap between 6 insectivorous species and seasons using a Sørensen Similarity Index and Minimum Hamming Distances. For Sørensen Similarity Index value equal to 0 have no dietary overlap and values at 1 have full dietary overlap. For Hamming Distances values range from 0 (all common diet choices) to 616 (no common diet choices). Species are denoted by the first letters of their genus and species name. The number next to the species denote the season it was collected in, 1 (wet season) and 2 (dry season). Dietary breath shows the total number of unique genetic sequences found in the diet.

<table>
<thead>
<tr>
<th>Species</th>
<th>Dietary Breath</th>
<th>Sørensen Similarity Index (QS)</th>
<th>Minimum Hamming-Distances</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mw1</td>
<td></td>
<td>0.4</td>
<td>Mw2 99</td>
</tr>
<tr>
<td>Mw2</td>
<td>99</td>
<td>0.02</td>
<td>Tb1 93</td>
</tr>
<tr>
<td>Tb1</td>
<td>93</td>
<td>0.07</td>
<td>Tb2 112</td>
</tr>
<tr>
<td>Tb2</td>
<td>112</td>
<td>0.09</td>
<td>Pp1 180</td>
</tr>
<tr>
<td>Pp1</td>
<td>180</td>
<td>0.09</td>
<td>Pp2 157</td>
</tr>
<tr>
<td>Pp2</td>
<td>157</td>
<td>0.15</td>
<td>Pm1 162</td>
</tr>
<tr>
<td>Pm1</td>
<td>162</td>
<td>0.15</td>
<td>Pm2 140</td>
</tr>
<tr>
<td>Pm2</td>
<td>140</td>
<td>0.15</td>
<td>Mm 104</td>
</tr>
<tr>
<td>Mm</td>
<td>104</td>
<td>0.15</td>
<td>Mb 116</td>
</tr>
<tr>
<td>Mb</td>
<td>116</td>
<td>0.15</td>
<td></td>
</tr>
</tbody>
</table>
Figure 1: PCA of habitat preference of 4 insectivorous bats in Jamaica. Species falling to the right of the PC1 axis are found in cluttered environments and species to the left are in open environments. Species found to the top of the PC2 axis are found in edge environments.

80x80mm (300 x 300 DPI)
Figure 2: Temporal activity patterns of species throughout the night at their most preferred sites. Periods represented by the green checkered pattern are sites that have average activity level compared to activity at all other sites. Red, green, or blue represent periods of high, typical or low (respectively) activity compared to activity at all other sites and times.

128x149mm (300 x 300 DPI)
Figure 3: A schematic of prey species consumed by bats in this ensemble. In the absence of a reference database, identifications have been made by BLAST score and are limited to hypothesis at the order level (see supplemental bioinformatics section). Values at nodes or tips represent the number of MOTU assigned. The proportion of MOTU assigned by BLAST to a given taxonomic node for each predator is given by the pie chart.
Supporting Information: Technical specifications for acoustic and molecular protocols.

Acoustic Monitoring:

The microphone array was based on designs by Surlykke et al. [13]. Using eight Avisoft Bioacoustic CMPA microphones (Avisoft Bioacoustics, Berlin, Germany) attached to two Avisoft UltraSoundGate 416 interfaces (Avisoft Bioacoustics, Berlin, Germany), connected to a Dell PP04X laptop computer.

Diet analysis:

We extracted DNA using the QIAmp DNA Stool Mini Kit (Qiagen, UK) following the manufacturer’s instructions and the modifications suggested by Zeale et al. (2011). In addition, we used half of an InhibitEX tablet for each sample extended the first centrifuge step (Zeale step 4) to 3 minutes further pellet the particulate material. We stored the extracted DNA at -20°C prior to DNA amplifications. We amplified each sample using fusion primers designed for the Roche FLX sequencer as described by Bohmann et al. (2011) and based on the primers ZBJ-ARTF1c and ZBJ-ArtR2c described by Zeale et al. (2011).

We conducted PCR reactions as described by Bohmann et al. (2011) in a 20µl reaction containing 2µl of template DNA and using Qiagen multiplex PCR kits (Qiagen, UK) with the following modifications, we did not use Q solution (from the kit) or BSA (as suggested by Bohmann et al. 2011). Sequencing of the product was performed at the Liverpool Center for Genomic Research (University of Liverpool) using a ¼ plate, Lib-L chemistry on a Roche 454 GS FLX+ sequencing system (Roche Applied Sciences).
We analyzed sequences using the Galaxy platform (https://main.g2.bx.psu.edu/root, Goecks et al. 2010, Blankenberg et al. 2010, Giardine et al. 2005). We screened all recovered sequences for rare haplotypes (represented by <2 copies) and sequences much longer (>250bp) or shorter (<150bp) than expected length (230bp amplicon+primer). We removed primers and MID codes (see Clare et al. 2013 in press figure 1 for MID coding of sequencing). We collapsed all sequencing reads to unique haplotypes. We aligned the remaining haplotypes using clustal W in Bioedit (T. Hall, http://www.mbio.ncsu.edu/bioedit/bioedit.html) and edited the alignment manually using a known insect reference sequence. We clustered the sequences into molecular operational taxonomic units in the program jMOTU (Jones et al. 2011) and tested thresholds from 1-10bp. A 6bp threshold was selected to minimize over-splitting of MOTUs without losing taxonomic diversity (see Razgour et al. 2011).

We extracted representative sequences for each MOTU using PostgresSQL. We compared these representative sequences for each MOTU to a database of COI sequences retrieved from Genbank (http://www.ncbi.nlm.nih.gov/genbank/) October 2012. We used a basic local alignment search (BLAST) of this database to retrieve BLAST scores (e-value cut-off 0.0001). These scores were visualized in MEGAN (Huson et al. 2011) using default settings and a “Min Score” of 1. Hits were restricted to ordinal-level taxonomy even when additional detail was available.

We calculated the Minimum Hamming distances and the Sørensen Similarity Index to compare similarities in diet among seasons and species.
Sequencing results:

<table>
<thead>
<tr>
<th>Species</th>
<th>Season</th>
<th>Raw Sequences</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Macrotus waterhousii</em></td>
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<td>15103</td>
</tr>
<tr>
<td><em>Macrotus waterhousii</em></td>
<td>Early</td>
<td>16150</td>
</tr>
<tr>
<td><em>Tadarida brasiliensis</em></td>
<td>Late</td>
<td>11968</td>
</tr>
<tr>
<td><em>Tadarida brasiliensis</em></td>
<td>Early</td>
<td>9764</td>
</tr>
<tr>
<td><em>Pteronotus parnellii</em></td>
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<td>11999</td>
</tr>
<tr>
<td><em>Pteronotus parnellii</em></td>
<td>Early</td>
<td>11392</td>
</tr>
<tr>
<td><em>Pteronotus macleayii</em></td>
<td>Late</td>
<td>9861</td>
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<tr>
<td><em>Pteronotus macleayii</em></td>
<td>Early</td>
<td>10146</td>
</tr>
<tr>
<td><em>Molossus molossus</em></td>
<td>Late</td>
<td>11269</td>
</tr>
<tr>
<td><em>Mormoops blainvillii</em></td>
<td>Late</td>
<td>11449</td>
</tr>
</tbody>
</table>

References


