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1 Resource Partitioning by Insectivorous Bats in Jamaica

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14

15 **Key words:** diet, echolocation, habitat use, wing morphology

16

17 **Running head:** Resource Partitioning by Insectivorous Bats

18

19 **Abstract**

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

36

37 Introduction

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

49 For insectivorous bats, wing morphology and echolocation can be two major factors
50 associated with niche partitioning that may interact with prey consumption. Insectivorous bats
51 with short rounded wings appear adapted to forage in edge situations, while those with longer,
52 more pointed wings forage in more open habitat (Aldridge & Rautenbach 1987; Norberg &
53 Rayner 1987). For operation in clutter, areas where many echoes rebound from vegetation, wing
54 shape may be a less important determinant of habitat use than echolocation and ability to detect
55 prey (Aldridge & Rautenbach 1987). Bats use two main approaches to echolocation. Low duty-
56 cycle echolocators separate outgoing pulse from returning echoes in time while high duty-cycle
57 echolocators separate them in frequency (Fenton et al. 2012).—Some bats that use low duty-cycle
58 echolocation produce narrowband search phase echolocation calls and hunt in the open (Aldridge
59 & Rautenbach 1987; Schnitzler et al. 2003). Other low duty cycle species use calls dominated by

60 broadband frequency modulated sweeps, and tend to forage in edge situations (Aldridge &
61 Rautenbach 1987; Schnitzler et al. 2003). Bats using high duty-cycle echolocation produce
62 echolocation calls dominated by a constant frequency and are well suited to detecting fluttering
63 targets in clutter (Fenton et al. 2012). Some low duty-cycle bats that produce low intensity,
64 multiharmonic broadband signals hunt in cluttered settings, detect prey by sounds the prey
65 produce (Bell 1985) and more often hunt in cluttered situations. In general, for low duty-cycle
66 echolocators, longer search phase signals that are narrower in bandwidth give more effective
67 operational range than shorter, broadband signals (Simmons and Stein 1980).

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

79 We use modern and traditional approaches to assess the potential for resource partitioning
80 among six sympatric species of insectivorous bats that roost and forage in the same area.

81 Specifically, we used flight path reconstructions and molecular analysis of diet along with
82 traditional measures of wing morphology and echolocation calls. The six species we studied

83 | include *Pteronotus parnellii* (Fig. 1) (Gray 1843), *Pteronotus macleayii* (Gray 1843), *Mormoops*
84 | *blainvillii* Leach 1821 (Mormoopidae), *Macrotus waterhousii* Gray 1843 (Phyllostomidae),
85 | *Tadarida brasiliensis* (I. Geoffroy, 1824) and *Molossus molossus* (Pallas 1766) (Molossidae)).
86 | A seventh species, *Pteronotus quadridens* (Gundlach 1840), was included in analyses of flight
87 | behaviour, wing morphology and echolocation call characteristics but inadequate sample sizes
88 | precluded analysis of its diet. We tested the prediction that species-specific characteristics (e.g.,
89 | wing morphology and echolocation behaviour), traditionally used to infer mechanisms of
90 | resource partitioning are reflected by quantifiable differences in habitat use and diet.

91

92 | **Methods**

93 | We worked in Jamaica near Windsor Cave (18°21'N, 77°38'W, elevation 100-500 m), a
94 | large daytime roost for the above-referenced species, except for *M. molossus* which roosts in
95 | houses in the study area. An additional insectivore, *Chilonatalus micropus*, roosts in the cave
96 | but was rarely encountered in our work. Land use is a mix of disturbed closed-canopy
97 | evergreen broadleaf forest, agriculture fields of sugar cane, pasture, and coffee, and riparian
98 | forest along the banks of the Martha Brae river. According to Genoways et al. (2005), six
99 | additional insectivorous species occur in Jamaica, but they were not encountered in our work.
100 | We captured bats in mist nets and harp traps from 4 December 2010 – 2 April 2011, 13 July - 6
101 | August 2011, and 12 May - 9 June 2012. Following Aldridge and Rautenbach (1987), we
102 | recorded mass, species, sex, reproductive status, age, forearm length (fl), length of wing hand
103 | (lwh), length of arm wing (law) and body width (bw). We held bats individually in cotton bags to
104 | collect fecal samples from them. Fecal samples were frozen within 12 h of collection and bats
105 | were released within 60 min of capture. We photographed the right wing and tail membrane

106 against graph paper with a grid of 5 mm for individuals of each species. From these photographs
107 we calculated total surface area (S) and surface area of the hand wing (Shw) and arm wing
108 (Saw), wingspan (b), aspect ratio (AR), wing loading (WI), tip length ratio (TI), tip area ratio
109 (Ts) and tip shape index (I) (Norberg & Rayner 1987). For species captured all year, we divided
110 fecal samples based on collections made during dry (December 2010 to March 2011) and wet
111 (July to August 2011) seasons.

112 To assess habitat use, we deployed two four-microphone arrays (see Supplemental File)
113 to acoustically monitor 9 sites (minimum 5 nights each), representing cluttered, open, and edge
114 habitats (Schnitzler et al. 2003).—These sites were within a circle with a radius of 750 m.—The
115 first site (Site 1) for acoustic monitoring was the front yard of a home. Site 2 was a cliff face that
116 overlooked tree canopy. Site 3 was an area that had been cleared for cultivation but has since
117 been abandoned, It was composed mostly of ferns. Site 4 was a section of river located in a
118 cluttered habitat. Site 5 was a section of river located in an open habitat. Site 6 was the boundary
119 of a cluttered forest and an open pasture. Site 7 was a small patch of forest surrounded by roads
120 and open habitats. Site 8 was a sloped hillside along a forest trail. Site 9 was a forested plateau
121 located between the peaks of two hills. We recorded echolocation activity continuously from 50
122 minutes before to 790 minutes after sunset using Avisoft Recorder USG software (Avisoft
123 Bioacoustics, Berlin, Germany) with a 250 kHz sampling frequency, and 8 bit format. We used
124 callViewer18 (Skowronski & Fenton 2008) to assign echolocation calls to species. We used an
125 activity index (AI) (Miller 2001) to assess levels of bat activity by habitat. AI is based on the
126 number of one minute long files in which a species was detected, modified for relative habitat
127 use (species AI on a given night / total AI for the species). We used principal components
128 analysis (PCA) on relative AI to reduce dimensionality of habitat use and SaTScan (v.9.1.1;

129 SaTScan, Boston, USA) to compare activity levels between sites to identify periods of high and
130 low activity. SaTScan is designed to discover statistical significances of disease outbreaks
131 across space and time. The same principals used by the software to analyze the occurrence of
132 diseases can also be applied to determine peak activity (A. Adams, unpublished). This approach
133 allowed us to examine levels of activity across all sites and determining the probability that one
134 peak in activity was greater than peaks in other locations.

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

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

146

147 ***Statistical Analyses***

148 To determine morphological partitioning, we used independent sample Kruskal-Wallis
149 tests to compare morphological values among species with non-normal distributions. To
150 determine behavioural partitioning associated with flight speeds, we ran Conover-Inman test for
151 all possible pairwise comparisons of mean flight speeds between species which determined if

152 they were statistically the same. To assess partitioning by diet, we used the Sørensen Similarity
153 Index (McCune, Grace & Urban 2002) and Minimum Hamming Distances (Hamming 1950) to
154 compare diets among species and between seasons.

155

156 **Results**

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

168 Interpretation of the search phase echolocation calls (Table 2) of these species generally
169 supports morphological categorizations; *P. macleayii*, *P. quadridens* and *M. blainvillii* use
170 broadband calls which provide details about prey but at shorter range resolution suggesting
171 adaptation for foraging in edge situations (Table 2). *Tadarida brasiliensis* and *M. molossus* use
172 longer, narrowband signals well suited for foraging in the open. The high duty cycle
173 echolocation behaviour of *P. parnellii* suggests that it is well suited for hunting fluttering targets
174 (flying insects) in clutter (Lazure & Fenton 2011; Fenton, Faure & Ratcliffe 2012). *Macrotus*

175 *waterhousii* also should be most active in clutter because of low intensity echolocation calls, and
176 detection of prey through sounds they generate (Bell 1985).

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

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

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

197 |

198

199

200 **Discussion**

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

212 Our results are similar to those from other studies (e.g. Aldridge & Rautenbach 1987;
213 Mancina et al. 2012) that reported how differences in wing morphology and echolocation call
214 design could result in resource partitioning. We found that differences in wing morphology and
215 echolocation behaviour coincided with differences in details of flight behaviour, habitat use, and
216 diet. The net effect is resource partitioning through a combination of habitat, temporal shifts in
217 activity and diet. Importantly, our findings extend previous studies by considering multiple
218 (rather than two) dimensions of partitioning within a bat community (Kunz 1973; Razgour,
219 Korine & Saltz 2011, Nicholls & Racey 2006), and demonstrate partitioning even in heavily used
220 edge habitats. – Previous evidence that bats partition time in their patterns of habitat use has been

221 limited to areas where water is limiting (Razgour et al. 2011; Adams & Thibault 2006). A new
222 application of SaTScan allowed us to detect previously overlooked temporal partitioning, e.g. at
223 Site 6, one of the most active sites used by the most species, each species had a unique set of
224 high and low activity times with minimal overlap among them (Fig. 23).

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

231 *Pteronotus parnellii* and other species in this complex (Clare et al. 2013) are high-duty cycle
232 echolocators (like old world members of the families Rhinolophidae and Hipposideridae). High
233 duty cycle echolocation provides better detection of fluttering targets, particularly in cluttered
234 situations (Lazure & Fenton 2011; Fenton et al. 2012). The analysis in Fig. 34 suggests that *P.*
235 *parnellii* ate more moths than any of the other species in this community. ~~But,~~ The values at
236 nodes represent the number of species-level BLAST assignments for a given taxon. A high rate
237 of false positive assignments of COI at higher taxonomic levels such as tribe and family (Wilson
238 et al. 2011) has been observed but order level assignments may actually be relatively robust
239 under certain informatic protocols (Clare unpublished data). While this means that any one
240 assignment should be treated conservatively. ~~However,~~ higher node assignments in our analysis
241 likely translate into higher support for a given node as more independent assignments to the
242 same taxa decrease the likelihood of a false positive. This analysis suggests the importance of
243 Lepidoptera in the diet of *P. parnellii* but identification to the species-level requires a DNA

244 library of local species, something not yet available for Jamaica. *Pteronotus macleayii* consumed
245 the highest diversity of prey with assignments at the widest variety of nodes (Fig. 34) even
246 though our sample for this species was limited.

247 According to the competitive exclusion principle, two species coexist in a stable
248 environment only if they occupy niches that differ in some measure (Hardin 1960; Chesson
249 2000). We demonstrate how differences in morphology and echolocation behaviour coincide
250 with differences in habitat use and diet. ~~Although Our-our~~ data suggest partitioning by diet, we
251 did not perform exclusion experiments and ~~thus~~ have no evidence ~~of to suggest that~~ competition
252 ~~is the cause.~~ Even during the dry season when the diversity of insects was lower (216 species
253 versus 312 in the wet season) the diets of the bats showed minimal overlap. Prey availability may
254 not have been limiting for the bats we studied but could have lead to differentiation of niches in
255 the past even though it is not currently apparent (see also Andrianaivoarivelo et al 2006; Bell
256 1980; Fukui et al 2009).

257 Morphometric comparisons of faunas of insectivorous bats typically reveal a cluster of
258 similar species and a few that are distinctly different (e.g., Fenton 1972; Aldridge & Rautenbach
259 1987; Findley 1993). In other words whether there are 5 or >30 morphometrically similar taxa,
260 the distance to the nearest neighbour in a plot changes little, but the distance to farthest
261 neighbours is greater. In one example 14 sympatric species of bats ate mainly beetles and moths
262 (Fenton et al. 1998). ~~These~~ bats differed in morphology and echolocation behaviour as reported
263 by Aldridge & Rautenbach (1987), but lack of details about the insects they consumed meant no
264 support for resource partitioning. Our data suggest that analyses of communities of sympatric
265 species of insectivorous bats will show, often minor, differences in morphology and other
266 features that collectively result in partitioning. We have demonstrated how differences in wing

267 morphology and echolocation calls and behaviour correlate with differences in habitat use and
268 diet.

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

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

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375 **Figures legends:**

376

377 ~~Figure 1: Flying *Pteronotus parnellii*~~

378

379 | Figure 21: PCA of habitat preference of 4 insectivorous bats in Jamaica. Species are denoted by
380 | the first letters of their genus and species name. Species falling to the right of the PC1 axis are
381 | found in cluttered environments and species to the left are in open environments. Species found
382 | to the top of the PC2 axis are found in edge environments.

383

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

388

389 | Figure 34: A schematic hypothesis of the distribution of prey species consumed by bats in this
390 | ensemble. In the absence of a reference database, identifications have been made by BLAST
391 | score and are limited to hypothesis at the order level (see supplemental bioinformatics section).
392 | Values at nodes or tips represent the number of MOTU assigned to the node. The proportion of
393 | MOTU assigned by BLAST to a given taxonomic node for each predator is given by the pie
394 | 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).

Species	N	Mass (g)	Forearm Length (mm)	Aspect Ratio	Wing Loading (N/m ²)	Tip Shape Index	Average Flight Speed (m/s)	Predicted Habitat
<i>Pteronotus parnellii</i>	25	13.9±1.2 C	52.88±0.76 A	6.6±0.4 B	7.5±0.8 D	1.2±0.2 A	6.9±1.5 (17) B	Cluttered
<i>Pteronotus quadridens</i>	7	6.8±0.3 F	38.29±0.76 D	6.6±0.6 B	6.3±0.5 E	0.9±0.2 B	7.6±1.5 (14) AB	Edge
<i>Pteronotus macleayii</i>	9	7.1±0.5 F	43.04±0.79 B	7.1±0.7 B	5.9±0.9 E	1.2±0.2 A	7.5±1.5 (18) AB	Edge
<i>Mormoops blainvillii</i>	18	9.4±0.9 E	45.92±1.24 B	6.2±0.2 C	5.5±0.6 E	0.8±0.1 B	9.0±2.0 (11) A	Edge
<i>Molossus molossus</i>	21	18.7±1.2 B	38.17±0.75 D	8.2±0.5 A	17.3±1.7 A	0.6±0.1 C	-	Open
<i>Tadarida brasiliensis</i>	16	10.8±1.1 D	39.86±0.72 C	8.7±0.6 A	11.2±1.4 B	0.8±0.1 B	9.4±1.7 (8) A	Open
<i>Macrotus waterhousii</i>	20	20.8±1.7 A	53.19±1.13 A	5.8±0.4 D	8.8±0.8 C	1.4±0.4 6	-	Cluttered

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).

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

* 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.

		Sørensen Similarity Index (QS)									
		Mw1	Mw2	Tb1	Tb2	Pp1	Pp2	Pm1	Pm2	Mm	Mb
Minimum Hamming Distances	Mw1		0.4	0.03	0.02	0.15	0	0	0	0.02	0.05
	Mw2	99		0.02	0.06	0.20	0.05	0.05	0.04	0.07	0.09
	Tb1	93	124		0.07	0.05	0.08	0.09	0.03	0.13	0.02
	Tb2	112	139	87		0.09	0.03	0.13	0.08	0.06	0.11
	Pp1	180	199	179	191		0.09	0.12	0.03	0.07	0.1
	Pp2	157	180	126	151	229		0.16	0.1	0.13	0.09
	Pm1	162	185	129	140	228	173		0.15	0.11	0.07
	Pm2	140	167	115	128	226	163	160		0.08	0.04
	Mm	104	129	75	98	186	129	136	120		0.07
	Mb	116	141	99	108	196	149	156	140	104	
Dietary Breath	58	92	37	56	152	99	104	82	48	64	

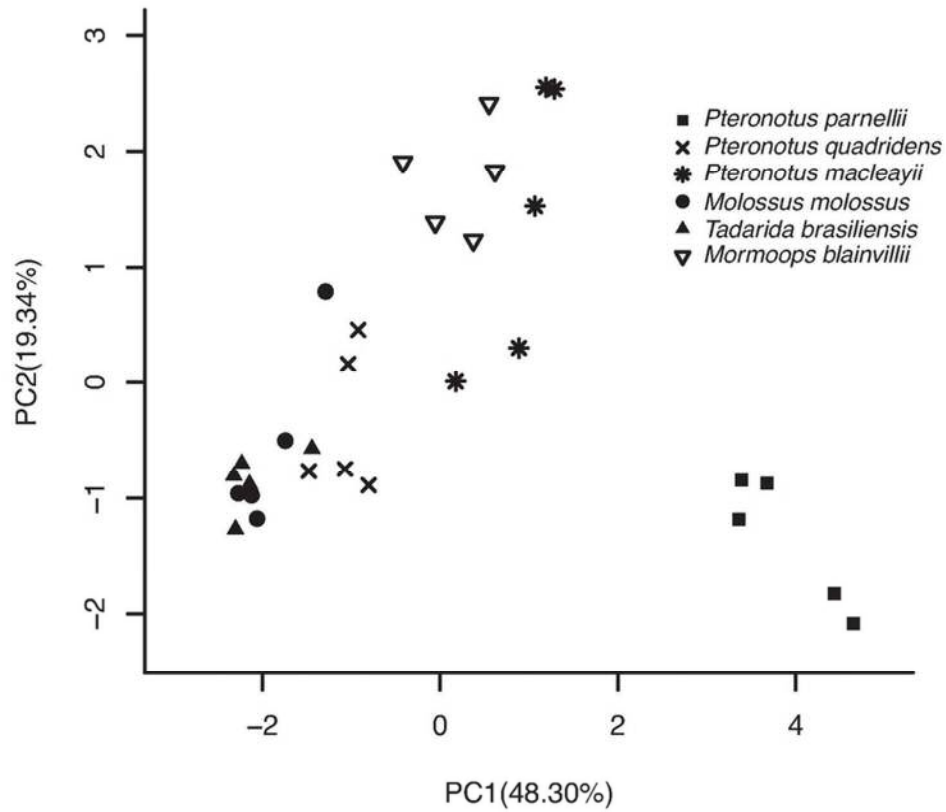


Figure 1: PCA of habitat preference of 4 insectivorous bats in Jamaica. Species falling to the right of the PC1 axis are found in cluttered environments and species to the left are in open environments. Species found to the top of the PC2 axis are found in edge environments.
80x80mm (300 x 300 DPI)

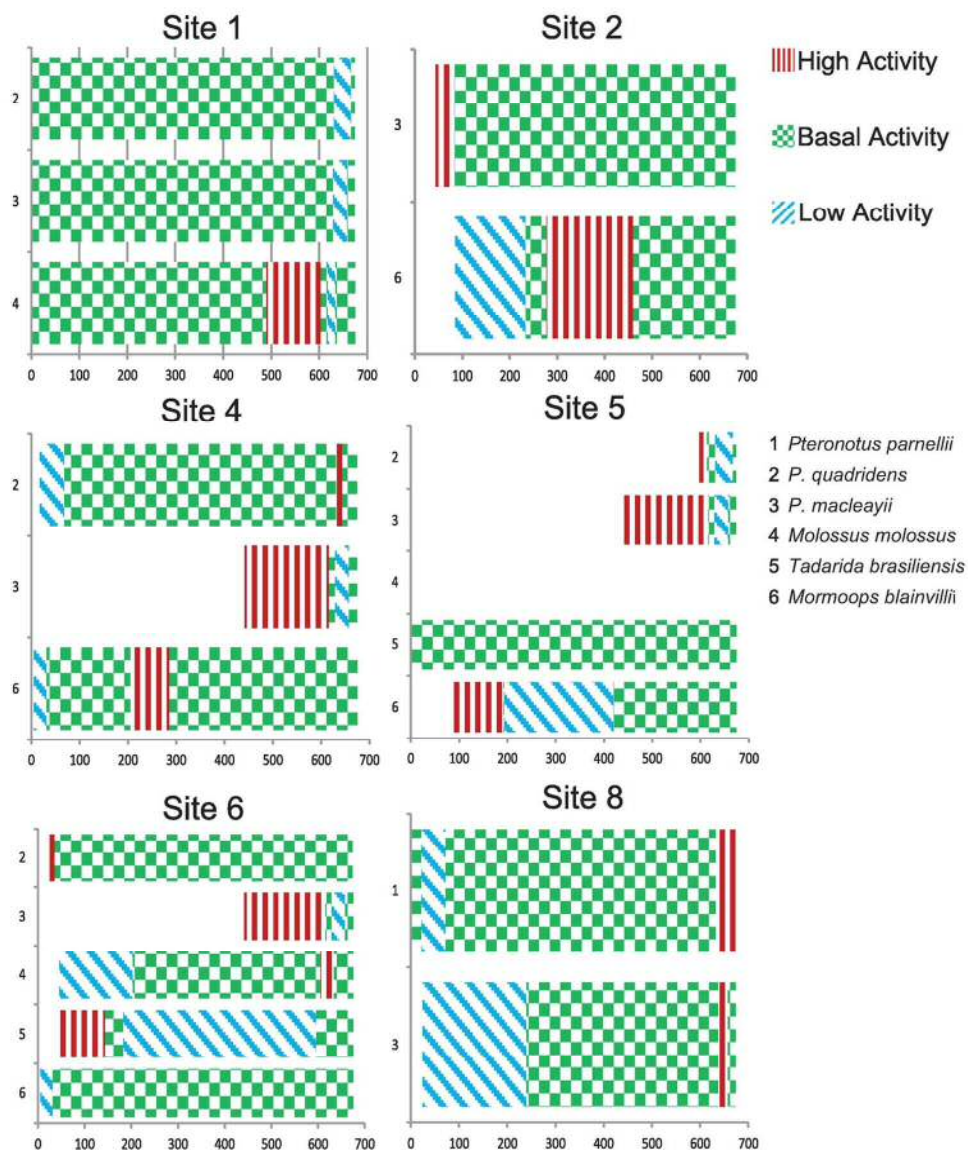


Figure 2: Temporal activity patterns of species throughout the night at their most preferred sites. Periods represented by the green checkered pattern are sites that have average activity level compared to activity at all other sites. Red, green, or blue represent periods of high, typical or low (respectively)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

128x149mm (300 x 300 DPI)

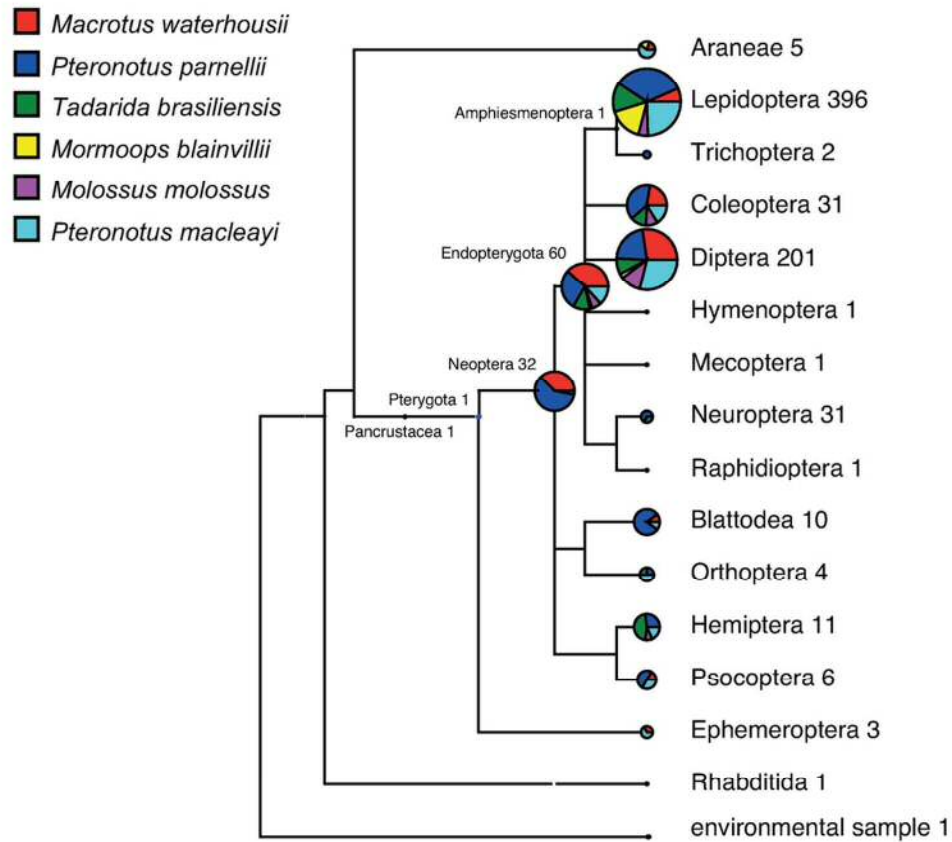


Figure 3: A schematic of prey species consumed by bats in this ensemble. In the absence of a reference database, identifications have been made by BLAST score and are limited to hypothesis at the order level (see supplemental bioinformatics section). Values at nodes and tips represent the number of MOTU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

98x88mm (300 x 300 DPI)



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 -20C 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 ($>250\text{bp}$) or shorter ($<150\text{bp}$) 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 PostgreSQL. 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:

Species	Season	Raw Sequences
<i>Macrotus waterhousii</i>	Late	15103
<i>Macrotus waterhousii</i>	Early	16150
<i>Tadarida brasiliensis</i>	Late	11968
<i>Tadarida brasiliensis</i>	Early	9764
<i>Pteronotus parnellii</i>	Late	11999
<i>Pteronotus parnellii</i>	Early	11392
<i>Pteronotus macleayii</i>	Late	9861
<i>Pteronotus macleayii</i>	Early	10146
<i>Molossus molossus</i>	Late	11269
<i>Mormoops blainvillii</i>	Late	11449

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