Resource Partitioning by Insectivorous Bats in Jamaica

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

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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 echolocation 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, echolocation 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 parnellii* ~~(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 (Brinkløv 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 ~~Supplemental~~ file) and calculated the number of molecular operational taxonomic units (MOTU) consumed by each species in each pooled sample (Bio-informatics in ~~supplemental~~ 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.

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

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, t~~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~~ our data suggest partitioning by diet, ~~-~~we did not perform exclusion experiments and thus have no evidence ~~of~~ 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 Andrianaivoarivelo 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

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

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Acknowledgements

We thank Gareth Jones and Collin Lazarus for use of laboratory space, Signe Brinkløv, Kristian Thygesen, Wayne Francis and Franklin Taylor for assistance in the field. We are grateful to Liam P. McGuire and John M. Ratcliffe for reading and commenting on an earlier version of this manuscript. This research was funded by a Discovery Grant to MBF and a post-doctoral fellowship to ELC both from the Natural Sciences and Engineering Research Council of Canada and a grant from the John D and Catherine T. MacArthur Foundation, administered by Windsor Research Centre. This research was conducted under permits issued by Jamaica's National Environment and Planning Agency to MBF and SEK (Ref. No. 18/27, dated 9 November 2010) and MAE (Ref. No. 18/27, dated 15 July 2011 and 9 May 2012). None of the authors has a conflict of interest associated with this manuscript.

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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 parnellii*~~

Figure ~~2~~1: 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 2~~3~~: 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 3~~4~~: 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).

Species	N	Mass (g)	Forearm Length (mm)	Aspect Ratio	Wing Loading (N/m²)	Tip Shape Index	Average Flight Speed (m/s)	Predicted Habitat
Pteronotus parnellii	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
Pteronotus quadridens	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
Pteronotus macleayii	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
Mormoops blainvillli	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
Molossus molossus	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
Tadarida brasiliensis	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
Macrotus waterhousii	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
Pteronotus parnellii	10	29.03±4.42	61.18±1.13	49.12±2.81	12.06±3.14	High	High	Cluttered
Pteronotus quadridens	10	4.49±0.792	80.03±1.43	60.84±1.51	19.19±2.26	Low	High	Edge
Pteronotus macleayii	10	4.80±1.21	70.65±1.81	54.69±1.15	15.97±1.99	Low	High	Edge
Mormoops blainvillii	10	2.95±1.13	66.65±1.87	44.09±3.64	22.56±4.08	Low	High	Edge
Molossus molossus	10	6.48±1.80	40.97±3.46	33.54±4.43	7.42±1.62	Low	High	Open
Tadarida brasiliensis	10	9.49±1.49	40.38±3.27	32.71±2.90	7.67±1.87	Low	High	Open
*Macrotus waterhousii**	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 nest 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

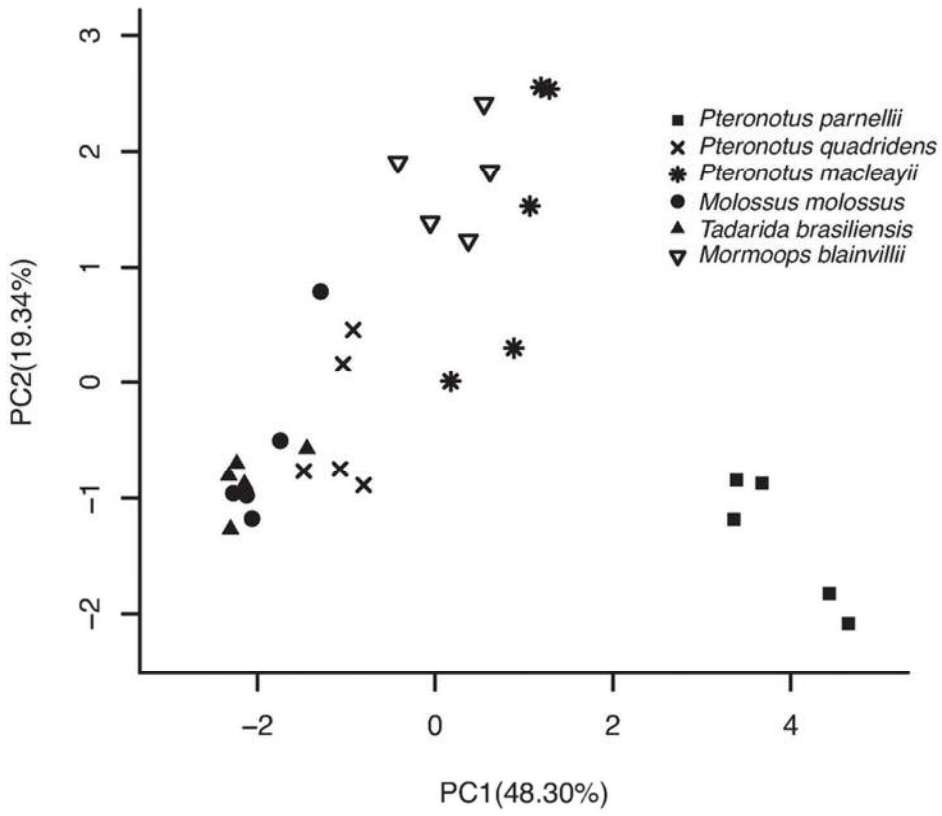

Figure 1: PCA of habitat preference of 4 insectivorous bats in Jamaica. Species falling to the right of the PC1 axis are found in cluttered environments and species to the left are in open environments. Species found to the top of the PC2 axis are found in edge environments.
80x80mm (300 x 300 DPI)

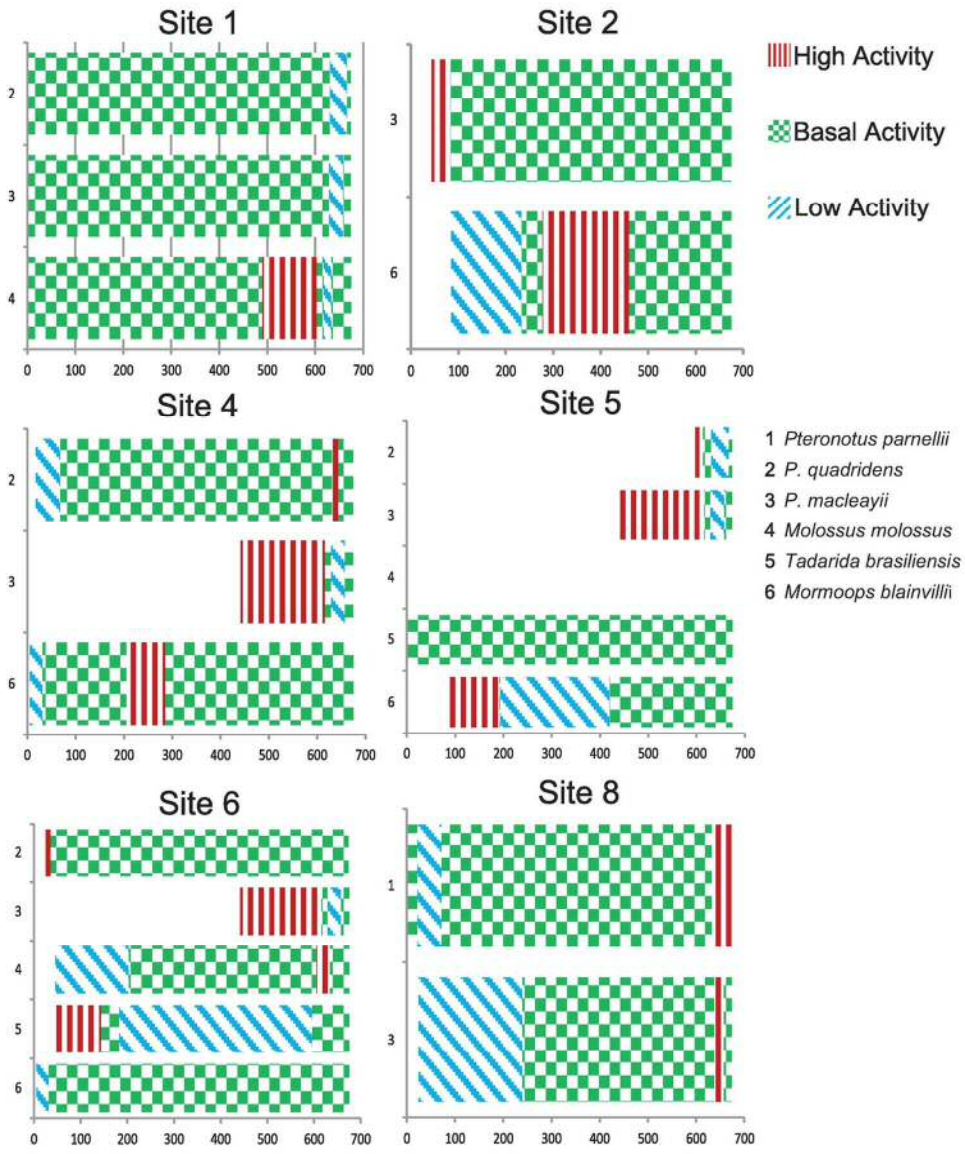

Figure 2: Temporal activity patterns of species throughout the night at their most preferred sites. Periods represented by the green checkered pattern are sites that have average activity level compared to activity at all other sites. Red, green, or blue represent periods of high, typical or low (respectively) activity compared to activity at all other sites and times.
128x149mm (300 x 300 DPI)

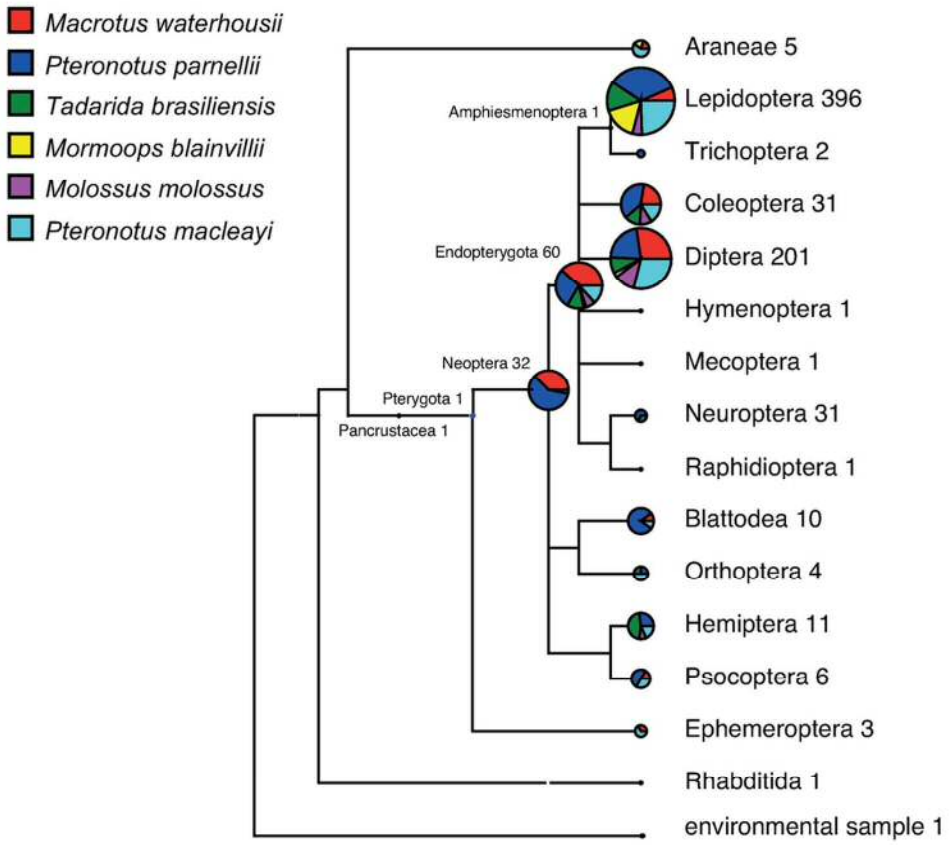

Figure 3: A schematic of prey species consumed by bats in this ensemble. In the absence of a reference database, identifications have been made by BLAST score and are limited to hypothesis at the order level (see supplemental bioinformatics section). Values at nodes or tips represent the number of MOTU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

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

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