

## Insectivorous bat activity at cenotes in the Yucatan Peninsula, Mexico

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The behaviour and habitat associations of aerial insectivorous bats are poorly understood despite constituting up to 65% of bat species in the Neotropics. In 2003, 2004 and 2005 we quantified the activity of insectivorous bats and their insect prey at pastureland and forest sites with and without cenotes (water-filled sinkholes) in the Yucatan Peninsula, Mexico. We used a time-expansion bat detector to survey each habitat for 24 nights and analysed 2,880 one-minute recorded sequences to determine bat activity. We identified 14 species and five phonic types belonging to four families. Bat activity and the average number of bat species acoustically sampled each night were significantly greater in habitats with cenotes than in those without. *Pteronotus personatus* and an unidentified molossid were recorded exclusively at cenotes. *Peropteryx macrotis* showed the highest activity of all bat species. In all habitats insects were more abundant during the rainy season but only in pastureland was bat activity significantly greater during the rainy season. Insect abundance was correlated with bat activity only at cenotes in pastureland. Cenotes are important foraging habitats for insectivorous bats as 16 species, 84% of those revealed by this study, were recorded feeding in these habitats and the number of feeding buzzes was higher in comparison to habitats without cenotes. Protection of cenotes and their surrounding vegetation should be a management priority in order to conserve the high diversity of insectivorous bats associated with these distinctive habitats.

*Key words:* Chiroptera, echolocation, insects, pastureland, time-expansion, water resources, Mexico

### INTRODUCTION

Although insectivorous bats represent nearly 65% of the bat fauna of the Neotropics (Simmons, 2005), information on their abundance, distribution, behaviour and habitat associations is scarce in comparison to species within other ensembles. The majority of insectivorous species are difficult to capture and most free-flying bats cannot easily be identified visually when in flight (Preatoni *et al.*, 2005), particularly in the tropics where there are many sympatric congeners. All microchiropteran insectivorous bats emit ultrasonic signals and process the returning echoes to detect, characterize and localize reflecting objects (Schnitzler and Henson, 1980). Bat detectors allow investigators to hear and record the ultrasonic calls of bats (Fenton, 1988), and therefore represent an important tool in their study. The analysis of echolocation calls can be used to identify species and to determine bat activity

acoustically (e.g., Vaughan *et al.*, 1997), although, the accuracy and reliability of this method have sometimes been controversial (Barclay, 1999; O'Farrell *et al.*, 1999).

Bat detectors have been used in the Neotropics to describe species' calls (O'Farrell and Miller, 1997, 1999; Rydell *et al.*, 2002; Vaughan *et al.*, 2004), produce more complete species inventories (Ochoa *et al.*, 2000; Barnett *et al.*, 2006), investigate foraging behaviour (Barclay *et al.*, 1981; Schnitzler *et al.*, 1994; Kalko and Condon, 1998; Thies *et al.*, 1998; Jung *et al.*, 2007) and examine the role of vocalisations in reproduction (Behr and von Helvesen, 2004). Fewer studies have used bat detectors to investigate habitat use by insectivorous bats, which depends not only on the wing morphology and echolocation characteristics of each species (Norberg and Rayner, 1987; Kalko and Schnitzler, 1998), but also on factors such as distance to clutter (i.e., foliage, water), weather

conditions, brightness of the moon, reproductive state, insect availability and predation pressure (Kaliko and Schnitzler, 1998; Downs and Racey, 2006).

Mexico has one of the most diverse bat faunas in the Neotropics with 138 recognised species (Ceballos, 2007). The country has a varied topography and diverse landscapes with habitats ranging from deserts to evergreen rainforest, which contribute to its high bat diversity. Seventy percent of the Mexican bat fauna is insectivorous (Medellín, 2003). Typically habitat use by insectivorous bats has been inferred from individuals captured in mist nets and harp traps. However, such methods are questionable due to their bias against insectivorous bats which are adept at avoiding such devices (Berry *et al.*, 2004), and many species presently go undetected (MacSwiney *et al.*, 2008). Bat detectors represent an alternative tool to investigate habitat use by insectivorous bats in Mexico, though, as yet, very few studies have adopted this approach (e.g., Rydell *et al.*, 2002; García, 2003; Estrada *et al.*, 2004; Avila-Flores and Fenton, 2005; Torres-Morales, 2007). Investigating which type or types of habitats bats use is of fundamental importance for effective conservation management. For example, in European countries such as the United Kingdom, most insectivorous species are under governmental protection, together with their preferred habitats such as broad-leaved woodland and water bodies (Racey, 1998a; Russ and Montgomery, 2002). We urgently need to determine which habitats are critical for insectivorous bat species in Mexico in order to conserve biodiversity.

A remarkable feature of the Yucatan Peninsula, Mexico, especially in the north, is the presence of cenotes. Cenotes (from the Mayan word 'dzonot') are water-filled sinkholes formed by the dissolution of limestone by carbonic acid and are the only visible signs of the distinctive underground aquatic system of the Yucatan Peninsula (Cervantes-Martínez *et al.*, 2002). Cenotes are numerous and represent the main source of fresh water for humans and wildlife. Water bodies, particularly those with calm surfaces, re-present important foraging sites for insectivorous bats (Racey 1998b; Ciechanowski, 2002; Russ and Montgomery, 2002) as the air above them is usually rich in insects (Rydell *et al.*, 1999). However until now there remains a paucity of information regarding the importance of cenotes for insectivorous bats. In this study, we investigated habitat use by insectivorous bats at four habitats in the Yucatan Peninsula, Mexico: cenotes in pastureland, cenotes in forest, and the same vegetation types but

without cenotes. We aimed to test the hypothesis that habitats with cenotes are utilised more by insectivorous bats, than those without.

## MATERIALS AND METHODS

### Study Area

Echolocation calls of insectivorous bats were recorded at eight sites: two cenotes in pastureland, two cenotes in the forest and two sites in each habitat type without cenotes (and an absence of water bodies within 4 km). Pastureland sites were located in the cattle zone east of Merida, capital of the state of Yucatan. This zone, originally semideciduous forest of medium-stature (Flores and Espejel, 1994), has been managed and totally modified by ancient civilizations and more recently has been managed for intensive production of henequen (agave: *Agave fourcroydes*), cultivated to produce rope and other fibre products (González-Iturbe *et al.*, 2002; Gómez-Pompa, 2003). Nowadays this area is also characterised by extensive cattle ranching with pastures of *Pennisetum purpureum*, *P. ciliare*, *Panicum maximum* and other grasses. The ranches also contain stands of secondary forest with different stages of succession and some scattered large trees such as *Ficus* spp., *Ceiba pentandra*, *Terminalia catappa* and *Brosimum alicastrum*. These trees have been allowed to remain as they provide shade and food for the cattle. Annual rainfall is 900–1,037 mm and average temperatures range from 25.9–26.4°C (Barber *et al.*, 2001). Sampling sites were 7–13 m above sea level. In pastureland sampling sites were Rancho Santa Teresa (21°10'N, 88°53'W) and Rancho Noyaxche (21°17'N, 88°36'W), and cenotes in pastureland were Cenote Azul (21°13'N, 88°40'W) and cenote Tortugas (20°54'N, 88°51'W) which are about 50–65 m in diameter with a surrounding vegetation belt of 30–50 m width. Dominant tree species are: *Ficus* spp., *Acacia pennatulata*, *Caesalpinia gaumeri*, *Lysiloma latisiliquum*, *Metopium brownii*, *Vitex gaumeri*, *Bursera simaruba* and *Gymnopodium floribundum*.

Forested sites were located in the north of the state of Quintana Roo within protected private biodiversity reserves. Vegetation type was mature semideciduous forest of medium-stature, more than 50 years old and characterized by trees of 15–20 m average height. Dominant tree species in the forested sites were: *Manilkara zapota*, *M. brownii*, *L. latisiliquum*, *Thrinax radiata*, *B. simaruba*, and occasional large individuals of *B. alicastrum*, *Ficus pertusa*, and *Acacia dolichostachya* (Schultz, 2005). Annual mean temperature is 23.7°C and rainfall is 1,124 mm (Barber *et al.*, 2001). Elevation was about 5–10 m above sea level. Forest sampling sites were Selva Rancho Santa María forest (21°06'N, 87°10'W) and El Eden forest (21°12'N, 87°12'W). Cenotes in forest were Cenote Benita (21°13'N, 87°12'W) and Cenote Km 4.5 (21°04'N, 87°12'W), which were 30–70 m in diameter. Dominant trees species in the vegetation surrounding cenotes were *M. zapota*, *Ficus* spp., *M. brownii*, *V. gaumeri* and *Haematoxylon campechianum*.

### Acoustical Sampling

Ninety-six nights of sampling were conducted in 2003, 2004 and 2005. Each of the eight localities was sampled for six nights during both the dry and rainy season. Sampling on

consecutive nights at the same location was avoided. Each night, echolocation calls were recorded with a Pettersson D980 bat detector (Pettersson Elektronik AB, Uppsala, Sweden) and stored using a Sony Walkman Professional WM-D6C recorder with TDK Type IV metal tapes. The bat detector was set to automatically record calls within 3 seconds of real time and time-expand them ( $10\times$ ) for a continuous period of 10 minutes. These recordings were stored on Channel 1 of the Walkman. At the same time, the bat detector was also tuned to frequency division, which output was recorded on Channel 2 of the Walkman. Call characteristics were measured only from the time expansion recordings. The first recording period was carried out 30 min after sunset and the two subsequent ones at hourly intervals. To minimize call variability and changes in detection due to habitat structure (Patriquin *et al.*, 2003), we recorded the calls in open space. In habitats with cenotes, the microphone was orientated towards the water and at the forested sites towards natural vegetation gaps. A tripod was used to maintain the bat detector at an angle of  $45^\circ$  from the horizontal at 1.3 m above ground (Avila-Flores and Fenton, 2005).

### Sound Analysis

To aid species identification and estimate bat activity, calls were analyzed with BatSound Pro 3.10 (Pettersson Elektronik AB, Uppsala, Sweden). We analyzed spectrograms using a sampling rate of 44100 Hz, Fast Fourier Transformation (FFT) size of 512. Only search-phase calls of sufficient intensity to permit analysis were considered. Each 10-min recording was divided into 1-min intervals (hereafter referred as 'files'). Because display settings affect the visual perception of calls, we used the same parameter levels for the spectrogram window (2,000-ms per plot; threshold = 10; amplitude contrast = 3 — Avila-Flores and Fenton, 2005). Exploratory analysis of the real time recordings (frequency division mode), demonstrated that high bat activity in some of our sites resulted in overlapped passes (where a pass is defined as two or more pulses emitted by a single bat — Fenton, 1970). This complicated the objective of counting bat passes, and prevented their use in assessing bat activity. Instead, we estimated bat activity with the acoustic activity index (AI) of Miller (2001). The AI is calculated by summing the number of one-minute time blocks (files) for which a certain species is detected as being present. The AI was calculated by analyzing the time-expansion recordings. We defined the 'presence' of a species when two or more clear pulses were visualized within a file. The AI provides a standardized method to compare each species' relative contribution to activity at multiple sampling sites or at a single site over time (Miller, 2001). We also counted the number of feeding buzzes (defined as an increase in pulse repetition rate associated with attempted captures of prey — Griffin *et al.*, 1960) to provide an indication of prey capture at each habitat.

### Species Identification

Recordings from free-flying bats were identified by comparing them with echolocation call libraries constructed during the present study. Mist-nets at ground level, harp traps and canopy nets were used to capture bats. We constructed an echolocation call library by recording the calls of captured individuals (identified to species) released from the hand about 6 m from the bat detector and  $> 8$  m from vegetation in the same open areas where we acoustically sampled free flying bats. Species caught and their calls recorded were: *Saccopteryx bilineata*,

*Pteronotus parnellii*, *P. davyi*, *Molossus rufus*, *Lasiurus ega*, *Myotis keaysi*, *Eptesicus furinalis* and *Rhogeessa aeneus*. *Nyctinomops laticaudatus* was recorded near a known roosting site. Identification of the remaining species was based on comparison of previously published descriptions of calls from echolocation studies. Identification of the calls of *Peropteryx macrotis*, *Mormoops megalophylla*, *Molossus sinaloae* and *Lasiurus intermedius* was based on previously published calls of individuals captured in the Yucatan (Granados, 2001; Rydell *et al.*, 2002), Belize (O'Farrell and Miller, 1997, 1999; Miller, 2003) and other Central American localities (Jung *et al.*, 2007). *Pteronotus personatus* was identified based on published data about its echolocation (O'Farrell and Miller, 1997, 1999). Although *Natalus stramineus*, a common insectivorous species that produces low intensity calls, was captured and recorded, we were unable to distinguish its calls reliably from those of phyllostomids flying near the microphone. Therefore, these calls were omitted from the acoustical analysis.

We conducted a discriminant function analysis (DFA) to assign confidence to identification of species using five call parameters: frequency of most energy, highest frequency, lowest frequency, duration and inter-pulse interval. DFA resulted in a 92.1% correct classification of the original data and 84.1% when cross-validated. Multivariate analysis of variance showed that the model was significantly different from random (Wilk's  $\lambda = 0.001$ ,  $F = 93.7$ ,  $P < 0.001$ ). Wilk's  $\lambda$  statistic and naive ranking showed that the duration of the call and frequency of most energy (FME) were the most important variables discriminating between species. The high level of correct classification of the calls identified confirmed that assessment of species by their echolocation calls was feasible (MacSwiney *et al.*, 2008).

### Insect Sampling

We estimated insect relative abundance using two methods: a light trap, and a Malaise trap (Alana Ecology Ltd.), bearing in mind that most insect sampling methods are biased towards certain groups of insects and that many variables (e.g., moonlight, wind, temperature) can affect the success of insect trapping (Leather and Watt, 2005). The light trap, fitted with a double lamp 43.5 cm long, emitting ultra-violet and white light, was hung from a tree branch 1.2 m from the ground in front of a vertical white sheet. The white sheet placed behind the light helped with collecting since it gives the flying insects a place to land and fewer escape routes. The light trap was switched on for 10-minute periods immediately after acoustical recordings. A plastic jar with a mixture of plaster of Paris and potassium cyanide was used to kill insects attracted by the light trap, which were then stored in paper bags. The Malaise trap was set at sunset and left open for an average of five hours. The collection bottles used on the trap were filled to about one third of their 500-ml capacity with 70% ethyl alcohol for preserving insect samples in the field. Insects collected by both methods were dried to constant weight and weighed with an analytical balance (AP310-OHAUS Corp.). The insect biomass obtained from both traps was pooled to evaluate insect abundance. Insects that were considered too large to be eaten by the bats species we encountered were eliminated from the analyses.

### Statistical Analysis

Data could not be transformed to fit a normal distribution and homogeneity of variances (Levene test), and non-parametric tests were used in subsequent analyses. An exploratory

analysis revealed no relationship between the time of recording and either the level of bat activity or the number of feeding buzzes (Kruskal-Wallis tests, both tests  $P > 0.05$ ). Thus, the three 10-min periods of recordings each night were pooled and each night of sampling was treated for the analyses as an independent sample. Differences in active files, number of feeding buzzes, and bat species richness among habitats were analyzed with the non-parametric Kruskal-Wallis test ( $H$ ). A post hoc Mann-Whitney  $U$ -test was used to identify pairs of habitats that were different (Dytham, 2003). To investigate seasonal patterns in bat activity, number of feeding buzzes and species richness among habitats we used the Wilcoxon's signed rank test ( $Z$ ). Finally, we used Spearman's correlation analysis to evaluate the relationship between insect abundance and bat activity. All statistical analyses were performed by using the statistical software SPSS 15.0 for Windows and the cut-off point for statistical significance was set at  $P = 0.05$ .

## RESULTS

### Insectivorous Bat Species Richness

In the echolocation recordings analyzed, we identified 14 species: *Peropteryx macrotis*, *Sacco-*

*pteryx bilineata*, *Mormoops megalophylla*, *Pteronotus parnellii*, *P. personatus*, *P. davyi*, *Molossus rufus*, *M. sinaloae*, *Nyctinomops laticaudatus*, *Eptesicus furinalis*, *Lasiurus ega*, *L. intermedius*, *Rhogeessa aeneus* and *Myotis keaysi*. Additionally we detected five molossid phonic types with similarities to echolocation calls of members of the genera *Eumops* and *Promops* (A. Guillén, personal communication — Fig. 1 and Table 1). The number of insectivorous bat species recorded per night among sites (averages: 6.3 at cenotes in pastureland, 2.7 in pastureland, 5.1 at cenotes in forest, and 0.75 in forest) was significantly different (Kruskal-Wallis;  $H = 55.8$ ,  $d.f. = 3$ ,  $P < 0.001$ ). A pair-wise comparison of insectivorous bats species richness between pastureland and forest with cenotes showed they were not significantly different (Wilcoxon;  $Z = -1.25$ ,  $n = 24$ ,  $P = 0.21$ ), but these habitats with cenotes had significantly more species than habitats without cenotes ( $U$ -test,  $P < 0.001$  in all cases). Only at cenotes in pastureland, was a significant

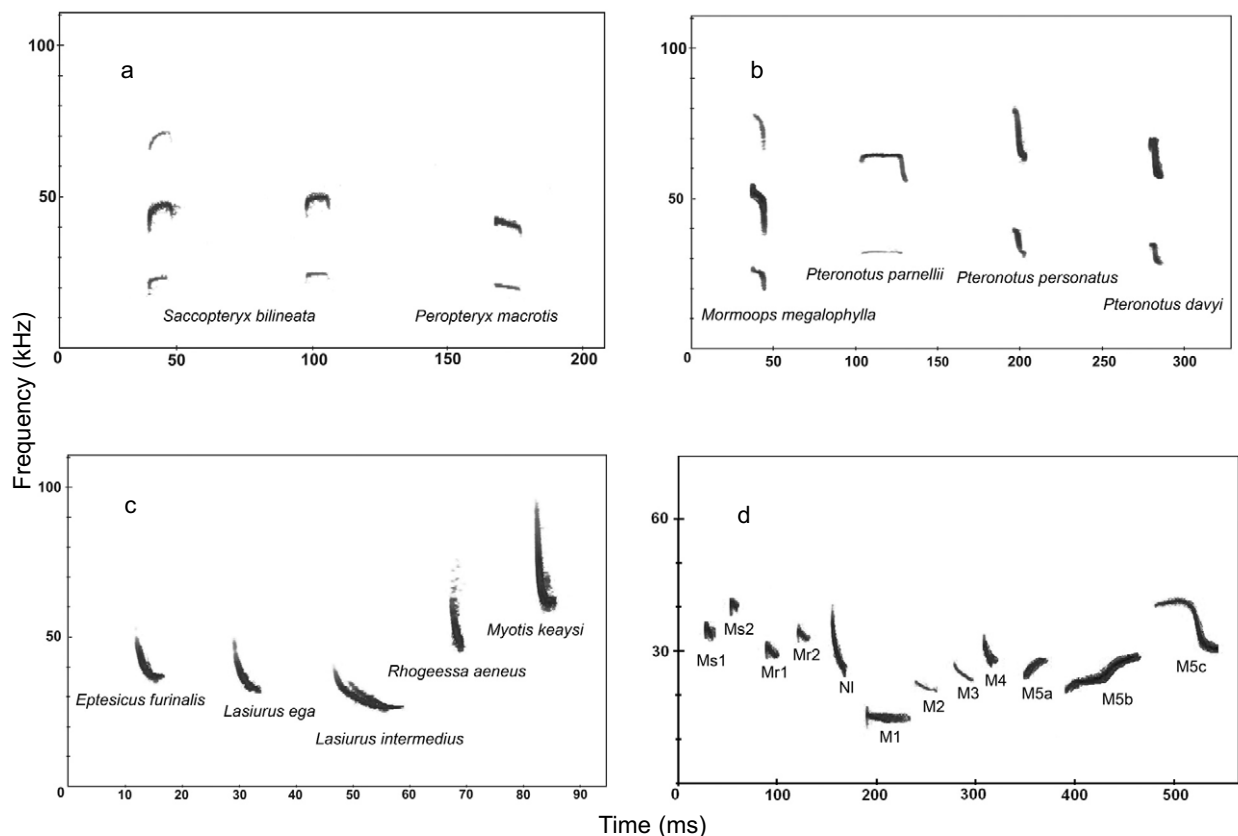


FIG. 1. Spectrogram showing the typical echolocation calls of the species and phonic types identified in the Yucatan Peninsula. (a) Emballonuridae, (b) Mormoopidae, (c) Vespertilionidae, (d) Molossidae: Ms1, low frequency call of *Molossus sinaloae*, Mr2, high frequency call of *M. sinaloae*; Mr1, low frequency call of *Molossus rufus*, Mr2, high frequency call of *M. rufus*; NI, *Nyctinomops laticaudatus*; M1, Molossid sp.1; M2, Molossid sp.2; M3, Molossid sp.3; M4, Molossid sp.4; M5a, short low frequency call of molossid 5; M5b, long low frequency call of molossid 5, and M5c = high frequency call of molossid 5. Note: The calls given for molossids are the most commonly observed of the many call types they emit

difference revealed between seasons in insectivorous bat species richness, with species richness being higher during the rainy season than during the dry one (Wilcoxon;  $Z = -2.81, n = 12, P < 0.05$ ).

*Bat Activity at Habitats and Between Seasons*

Out of the 2,880 files analyzed (equivalent to 48 h of recordings), 45.3% ( $n = 1,305$ ) showed bat activity. Habitats with cenotes had significantly greater bat activity than the same habitats without cenotes. Cenotes in pastureland had the greatest bat activity whereas forest had the lowest bat activity (Fig. 2 and Table 1). The number of feeding buzzes was significantly greater in habitats with cenotes than those without ( $U$ -test,  $P < 0.001$  in all cases — Table 1). No feeding buzzes were recorded in forest without cenotes (Fig. 2).

At cenotes in pastureland, bat activity was significantly greater during the rainy season than during the dry season (Wilcoxon;  $Z = -3.05, n = 12, P < 0.05$ ). In contrast, bat activity was significantly greater during the dry season at pastureland sites

without cenotes than during the wet season (Wilcoxon;  $Z = -1.95, n = 12, P < 0.05$ ). Feeding buzzes were often detected during the dry season (Table 1), but only at pastureland sites without cenotes was this difference statistically significant (Wilcoxon;  $Z = -2.03, n = 12, P < 0.05$ ).

*Insect Abundance*

Insect biomass differed among habitats (Fig. 3). Pastureland without cenotes had a significantly greater insect biomass than other habitats ( $U$ -test,  $P < 0.001$  in all cases). In contrast, the lowest insect abundance was recorded at cenotes in forest (Fig. 3). Insect abundance at pastureland sites without cenotes was significantly higher during the rainy season (Wilcoxon;  $Z = -2.35, n = 12, P < 0.05$ ), whereas at other habitats it was similar between seasons. Insect abundance was significantly correlated with bat activity only at cenotes in pastureland ( $r = 0.41, d.f. = 22, P = 0.04$ ) but not at other habitats. No correlation between insect abundance and the number of feeding buzzes was found (all correlations  $P > 0.05$ ).

TABLE 1. Number of files with activity recorded per habitat and season for each insectivorous bat species found in the north of the Yucatan Peninsula, Mexico. Numbers of feeding buzzes are presented in parentheses. Taxonomy of bat species follows Simmons (2005). cenpast: cenotes in pastureland; past: pastureland; cenfor: cenotes in forest; for: forest

Taxon	Habitat								Total
	cenpast		past		cenfor		for		
	Rainy	Dry	Rainy	Dry	Rainy	Dry	Rainy	Dry	
Emballonuridae									
<i>Peropteryx macrotis</i>	252 (118)	328 (251)	3 (0)	60 (19)	19 (1)	67 (69)	2 (0)	0 (0)	731 (458)
<i>Saccopteryx bilineata</i>	0 (0)	0 (0)	0 (0)	33 (8)	20 (9)	99 (66)	0 (0)	0 (0)	152 (83)
Mormoopidae									
<i>Mormoops megalophylla</i>	18 (1)	5 (0)	0 (0)	2 (0)	7 (1)	1 (0)	0 (0)	1 (0)	34 (2)
<i>Pteronotus personatus</i>	11 (1)	2 (0)	0 (0)	0 (0)	4 (0)	18 (2)	0 (0)	0 (0)	35 (3)
<i>P. parnellii</i>	29 (0)	3 (1)	3 (0)	0 (0)	16 (2)	14 (1)	3 (0)	0 (0)	68 (4)
<i>P. davyi</i>	50 (1)	106 (23)	1 (0)	4 (0)	23 (4)	27 (3)	3 (0)	2 (0)	216 (31)
Vespertilionidae									
<i>Eptesicus furinalis</i>	24 (1)	2 (0)	1 (0)	4 (0)	18 (0)	1 (0)	0 (0)	0 (0)	50 (1)
<i>Lasiurus ega</i>	87 (5)	4 (0)	1 (0)	0 (0)	13 (2)	17 (2)	0 (0)	0 (0)	122 (9)
<i>L. intermedius</i>	45 (4)	3 (0)	2 (0)	0 (0)	11 (0)	11 (0)	0 (0)	0 (0)	72 (4)
<i>Rhogeessa aeneus</i>	14 (0)	0 (0)	13 (2)	4 (0)	0 (0)	0 (0)	0 (0)	0 (0)	31 (2)
<i>Myotis keaysi</i>	10 (0)	12 (0)	5 (0)	4 (0)	42(11)	51 (7)	9 (0)	1 (0)	134 (18)
Molossidae									
<i>Molossus rufus</i>	7 (0)	8 (0)	1 (0)	1 (0)	0 (0)	0 (0)	1 (0)	0 (0)	18 (0)
<i>M. sinaloae</i>	7 (0)	0 (0)	0 (0)	0 (0)	9 (0)	0 (0)	1 (0)	0 (0)	17 (0)
<i>Nyctinomops laticaudatus</i>	85 (26)	16 (3)	2 (0)	12 (0)	19 (4)	3 (0)	2 (0)	0 (0)	139 (33)
Unidentified phonic types									
Molossid 1	3 (0)	3 (0)	0 (0)	0 (0)	0 (0)	82 (4)	0 (0)	0 (0)	88 (4)
Molossid 2	8 (0)	2 (1)	0 (0)	18 (2)	0 (0)	4 (0)	0 (0)	1 (0)	33 (3)
Molossid 3	19 (2)	34 (1)	4 (0)	22 (0)	2 (0)	0 (0)	0 (0)	0 (0)	81 (3)
Molossid 4	51 (2)	7 (1)	4 (0)	2 (0)	2 (0)	0 (0)	0 (0)	0 (0)	66 (3)
Molossid 5	22 (4)	2 (0)	0 (0)	2 (0)	0 (0)	0 (0)	0 (0)	0 (0)	26 (4)
Total	742 (165)	537 (281)	40 (2)	168 (29)	203 (34)	395 (154)	21 (0)	5 (0)	2111 (665)

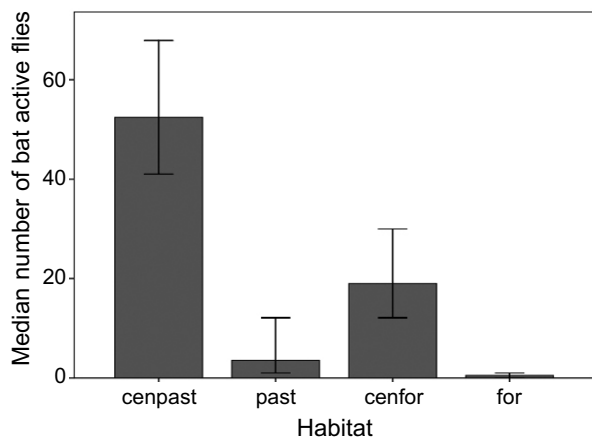


FIG. 2. Median values of bat activity recorded at habitats in the Yucatan Peninsula, Mexico. Errors bars represent  $\pm$  95% CI. cenpast: cenotes in pastureland; past: pastureland; cenfor: cenotes in forest; for: forest

### Habitat Preferences of Insectivorous Species

*Peropteryx macrotis* had the highest number of active files, accounting for 35% of the overall bat activity, whereas *M. sinaloae* had the lowest (Table 1). *Pteronotus personatus* and Molossid 1 (possibly *Eumops underwoodi*) were recorded exclusively at cenotes both in pastureland and forest. *Peropteryx macrotis*, *M. megalophylla*, *P. parnellii*, *P. davyi*, *N. laticaudatus* and *M. keaysi* occurred in all habitats, but had higher activity and number of feeding buzzes at cenotes than in habitats without cenotes (Table 1).

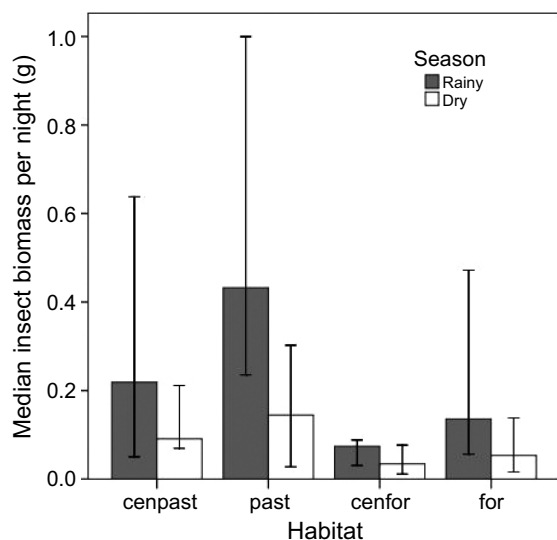


FIG. 3. Median values of insect biomass recorded at habitats of the Yucatan Peninsula, Mexico. Errors bars represent  $\pm$  95% CI. cenpast: cenotes in pastureland; past: pastureland; cenfor: cenotes in forest; for: forest

### DISCUSSION

We found higher activity of insectivorous bats at cenotes in pastureland and forest than in the same habitats lacking cenotes. Surveys carried out in European and North American countries have also shown that water bodies (e.g., ponds, lakes, rivers, streams) are the preferred habitats of many insectivorous bats, in comparison with landscape features that lack water (Fenton, 1970; Walsh and Harris, 1996; Vaughan *et al.*, 1997; Downs and Racey, 2006). The preference for cenotes by insectivorous bats may be explained by two main factors. First, bats need to drink water (Adams and Thibault, 2006). In the karstic landscape of the Yucatan which has no rivers or streams, cenotes represent the only source of drinking water for bats, so we can infer the importance of cenotes in satisfying their physiological needs for water. Secondly, water bodies with structured vegetation such as cenotes, attract bats because the vegetation concentrates insects, provides shelter from the wind (Verboom and Spoelstra, 1999) and possibly also offers protection from predators, particularly in the deforested agricultural landscape.

Cenotes were the most important foraging habitat for insectivorous species (considering the high number of feeding buzzes) in comparison with habitats without cenotes. We detected the foraging activity of 16 species of four families at cenotes. Paradoxically in all habitats fewer bat feeding buzzes were detected during the wet season, when insect abundance was highest. This contradiction may relate to the variation in specific groups of insects consumed by bats seasonally. For example, the higher number of feeding buzzes in the dry season could be due to bats (particularly emballonurids) feeding on smaller-bodied insects, which require a shorter time to find and process resulting in higher capture attempts per unit time (Racey *et al.*, 1998). In contrast, the lack of feeding buzzes of insectivorous bats from other families, suggests that during the dry season these species approach cenotes primarily to drink, or perhaps in some cases they are simply commuting. Further investigations are needed on the diet and foraging behaviour of insectivorous bats in the Yucatan in both seasons to explain our results. In general open spaces have lower densities of insects than along treelines (Watt *et al.*, 1997; Downs and Racey, 2006), however at our study sites we observed the opposite. These findings may partly be explained by the methods used to sample insects. Light traps in pastureland sites attracted a high

biomass of Lepidoptera and Coleoptera, probably because the vegetation did not attenuate the light intensity to the same extent as at our forested sites (C. MacSwiney, personal observation).

Cenotes had a higher number of aerial insectivore bat species each night than habitats without cenotes, with up to 12 detected at one cenote in a single night. The co-occurrence of many different insectivorous species at cenotes on the same nights suggests that one or more of the following may be true: (i) insect availability is high; or (ii) the large size of cenotes allows spatial partitioning, or (iii) different species have different temporal patterns of activity to avoid competition and optimize habitat-use. In Colorado, temporal partitioning was observed in five species of *Myotis* commuting to small water holes (2.5 m in diameter — Adams and Thibault, 2006). Although we do not have the data to assess temporal patterns of bat activity, our observations suggest that species such as *L. intermedius* are more active at sunset whereas *E. furinalis* are more active a few hours after sunset. These differences in the timing of habitat use by the insectivorous species at cenotes may facilitate resource-partitioning.

The low bat activity and absence of evidence of foraging by insectivorous bats in forest without cenotes was surprising. Sound transmission is affected by vegetation density, explaining the lower detection of bat species echolocating at frequencies below 40 kHz (Patriquin *et al.*, 2003). All the species recorded in forests without cenotes, with the exception of the phonic type Molossid 2, had echolocation call frequencies near or above 40 kHz, and were easier to detect among dense vegetation. It is possible that the calls of vespertilionids, which are short narrowband calls of low intensity (Jones and Teeling, 2006), may be attenuated by clutter at our sites, resulting in low detection. This is supported by the fact that some calls detected in the forest had to be discarded as they were not sufficiently intense to allow identification. Foraging behaviour may account for the low activity of molossids at forest without cenotes. Molossids are fast flyers that hunt in open, uncluttered space, above the forest canopy and rarely in small forest gaps (Crome and Richards, 1988; Kalko, 2001).

Cenotes are important habitats for threatened species in the Yucatan such as the carnivore *Chrotopterus auritus* and the gleaning insectivore *Micronycteris schmidtorum* (MacSwiney *et al.*, 2007). Our results suggest that two additional species seem to be associated with cenotes, *P. personatus* and the phonic type Molossid 1, which

might correspond to *E. underwoodi* (MacSwiney *et al.*, 2008). Both species are poorly documented in the Yucatan Peninsula, but previous records have been made at water bodies (Sánchez and Romero, 1995; García, 2003; MacSwiney *et al.*, 2003). Autecological studies using radio transmitters attached to these two species might clarify whether cenotes represent a critical habitat for their conservation.

Cenotes communicate with extensive underground water systems which have great potential for contamination because of the presence of numerous fractures and solution cavities that permit rapid infiltration (Pacheco *et al.*, 2001). Water contamination due to disposal of untreated domestic and animal waste and nitrogen-rich fertilizer used in agriculture are the major threats to cenotes (Pacheco *et al.*, 2001). Contamination of water bodies in other countries has resulted in a reduction of activity and feeding buzzes of some insectivorous species, an increase in populations of opportunistic species, and the death of hundreds of bats intoxicated with alkaloids produced by blooms of blue-green algae (Racey, 1998b). Our results indicate that cenotes represent a key habitat for bats, concentrating activity and providing food resources for many insectivorous species. Conservation of the water and the surrounding vegetation of cenotes, particularly in the agricultural landscape, is a high priority for future biodiversity action plans.

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