Cambodian bat echolocation: a first description of assemblage call parameters and assessment of their utility for species identification

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មូលន័យសង្ខេប

ពពួកសត្វប្រចៀវបង្កបានជាសមាសភាគសំខាន់នៃថនិកសត្វចំរុះនៅតំបន់អាស៊ីអាគ្នេយ៍ និងជាសូចនាករមានសក្តានុពលនៃផលប៉ះពាល់ជីវៈចម្រុះទូលំទូលាយ ដែលបណ្តាលមកពីការបាត់បង់ទីជម្រក និងការប្រែប្រួលអាកាសធាតុ។ ការអភិវឌ្ឍវិធីសាស្ត្រ ដែលមានប្រសិទ្ធភាពសម្រាប់ការធ្វើបញ្ជីសារពើភណ្ឌនិងការសង្កេតតាមដានប្រចៀវនៅអាស៊ីអាគ្នេយ៍ គឺមានសារៈសំខាន់ណាស់ប្រសិនបើតម្រូវការអភិរក្សនឹងត្រូវបានកំណត់ និងសក្តានុពលជាជីវៈសូចនាករនឹងត្រូវបានដឹង។ ដើម្បីទទួលបានលទ្ធផលនេះ យើងផ្តល់ជូននូវការពណ៌នាដំបូងពីសម្លេងដៅកន្លែងតាមរយៈពេល (Time-expanded echolocation calls) ពីសំណុំប្រចៀវនៅកម្ពុជា ដែលរួមមាន១៧ប្រភេទពីឧទ្យានជាតិភ្នំគូលែន។ យើងនឹងវាយតម្លៃភាពគួរឱ្យជឿបាននៃវិធីសាស្ត្រកំណត់សម្លេងសម្រាប់រកអត្តសញ្ញាណប្រចៀវ១៣ប្រភេទនៃប្រភេទប្រចៀវទាំងនោះ។ ការវិភាគមុខងារខុសៗគ្នានៃ៤២៨សម្លេងដៅកន្លែង (Echolocation calls) ដែលត្រូវបានបញ្ចេញដោយប្រចៀវ១៣ប្រភេទ បានបង្ហាញថាការកំណត់អត្តសញ្ញាណដោយសម្លេងគឺគួរឱ្យជឿជាក់បានក្នុងកាលៈទេសៈភាគច្រើនតាមរយៈការចាត់ថ្នាក់យ៉ាងត្រឹមត្រូវលើ៨៥%នៃសម្លេងដៅកន្លែង។ គំរូល្អបំផុតទាំងនោះគឺអាស្រ័យលើប៉ារ៉ាម៉ែតសម្លេងពីរនិងមានសារៈសំខាន់ណាស់ក្នុងន័យស្ថិតិ។ ការសិក្សាបន្តលើការធ្វើឯកសារជាភូមិសាស្ត្រនិងប្រភពផ្សេងទៀតនៃការប្រែប្រួលសម្លេងដៅកន្លែងទាំងនោះ ដែលត្រូវបានបញ្ចេញដោយសត្វប្រចៀវនៃប្រទេសកម្ពុជា គឺចាំបាច់សម្រាប់ជួយសម្រួលដល់ការអភិវឌ្ឍរបៀបប្រមូលគំរូតាងសម្លេង ដែលជាឧបករណ៍សម្រាប់ការអភិរក្សប្រចៀវទាំងនោះ។

Abstract

Bats form a major component of mammal diversity in Southeast Asia and are potential indicators of wider biodiversity impacts resulting from habitat loss and climate change. The development of effective methods for inventorying and monitoring Southeast Asian bats is critical if their conservation needs are to be determined and their potential as bioindicators realised. To this end, we provide the first description of time-expanded echolocation calls from a Cambodian bat assemblage comprising 17 species from Phnom Kulen National Park. We further evaluate the reliability of acoustic methods for identifying 13 of these taxa. Discriminant function analysis of 428 echolocation calls produced by the 13 bat species indicated that acoustic identification was feasible in most instances by correctly classifying 85% of calls. The best models relied on two call parameters and were statistically significant. Further studies documenting geographical and other sources of variation in the echolocation calls produced by Cambodia's bat fauna are necessary to facilitate development of acoustic sampling as a tool for their conservation.

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Keywords
Acoustic sampling, bat species identification, Phnom Kulen National Park.

Introduction

Bats form a major component of the Southeast Asian mammal fauna, constituting approximately 30% of the region's mammal species and as many as half of all mammal species in the tropical rainforest ecoregions (Kingston, 2010). This group provides economically significant ecosystem services in plant pollination, seed dispersal and arthropod suppression (Kunz *et al*., 2011). Bats also possess a variety of traits that support their use as bioindicators, reflecting wider biodiversity impacts from habitat loss and climate change (Jones *et al*., 2009). Like much of the Southeast Asian fauna, however, bats are severely threatened, with only 18% of species populations in the region presently considered stable by the International Union for the Conservation of Nature (IUCN) (Kingston, in press).

The global success, species richness and ability of bats to exploit diverse niches are largely due to their capacity for powered flight and echolocation (Jones & Teeling, 2006). Echolocation entails the use of reflected sound waves whereby bats use the difference between the sounds they produce and the returning echoes they hear to collect information about their surrounding environment. This acoustic process is largely ultrasonic and allows bats to navigate complex three-dimensional spaces in complete darkness. Echolocation tasks exert a strong selective pressure on signal design, favouring species-specific signal types linked to ecological conditions (Schnitzler *et al*., 2003). As a consequence, once adequate reference recordings have been obtained from bats of known identity, these can be used to identify species exclusively by their calls (Brigham *et al*., 2004).

The development of effective methods for inventorying and monitoring bat populations is essential if their conservation needs are to be determined and their potential as bioindicators realised. Because traditional sampling methods for bats—mist nets and harp traps—are rarely employed more than a few metres above ground level in surveys in Asia, they typically fail to capture species that habitually fly in open areas and/or above the forest canopy, even in the most intensive studies. Detecting bats from their calls is widely viewed as a means of overcoming these limitations (Brigham *et al*., 2004). Recent studies indicate that acoustic identification of Southeast Asian bat species is feasible and that acoustic methods are indispensable for maximising sampling completeness in field surveys (Furey *et al*., 2009; Hughes *et al*., 2010, 2011), an essential requirement for effective conservation planning.

Despite this, acoustic sampling has been rarely employed in continental Southeast Asia to date and detailed descriptions are lacking for the echolocation calls of most bat species in the region. We address this by providing the first description of time-expanded echolocation calls for an assemblage of Cambodian bats and evaluate the reliability of acoustic methods for species identification. The study was undertaken at Phnom Kulen National Park as part of a series of ongoing bat surveys that primarily rely on harp traps and mist nets in this area. As Cambodia's bats are poorly known relative to neighbouring countries (Kingsada *et al*., 2011; Ith *et al*., 2011a), our overall purpose was to provide information to assist future bat research and conservation efforts in the country.

Methods

Study site

Phnom Kulen National Park is in Siem Reap Province, Northwest Cambodia (Fig. 1). The region has a tropical monsoon climate with a mean annual rainfall of 2,050 mm and an average annual temperature of 24°C (Hou *et al*., 2004). The national park covers an area of 37,350 hectares and encompasses lowland areas and sandstone hills that culminate in two plateaus reaching 450 m above sea level (a.s.l.). Habitats include evergreen and semi-evergreen forests on hillsides and plateaus, while lowland areas were originally dominated by dry dipterocarp forest, of which only small, degraded areas now remain (Neou *et al*., 2008).

Capture methods and species identification

Thirty-two nights of sampling were undertaken in semi-evergreen forest of variable condition within the Kbal Spean area (13°36′22′′N, 104°00′96′′E) of the national park between April and July 2010. Live-trapping was carried out from 18:00–21:00 h each night using a four-bank harp trap (capture surface: 2.4 m^2) and 70 denier mist nets (capture surface: 30 m^2), giving a total sampling effort of 234 m^2 harp-trap-

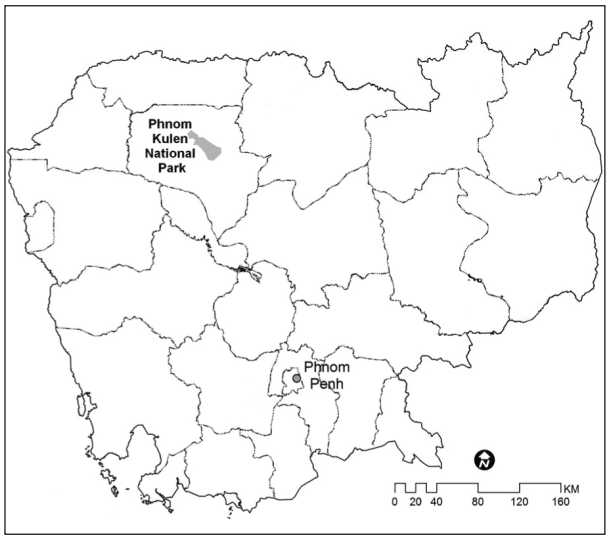

Fig. 1 Location of Phnom Kulen National Park in Cambodia.

hours and 2,889 m^2 mist-net-hours. A single night of sampling was also undertaken from 18:00–19:30 h using a harp trap and mist net (capture surface: 15 m^2) at a cave entrance in a forest area (13°67′74″N, 104°02′01″E, entrance altitude 183 m a.s.l.) in June 2010. Sampling was avoided on consecutive nights at the same location.

All bats captured were measured, photographed and identified in the field using Borissenko & Kruskop (2003) and Francis (2008). Where necessary to confirm species identifications, a minimum number of non-reproductively active individuals were retained as voucher specimens. All other bats were released as near as possible to their capture site. Skulls and bacula (where taxonomically important) of voucher specimens were subsequently examined and all specimens were deposited at the Centre for Biodiversity Conservation Zoological Collection at the Royal University of Phnom Penh. A full list of specimen material examined is given in Annex 1. Nomenclature follows Simmons (2005), with some modifications (Soisook *et al*., 2008).

Acoustic methods and call measurement

Time-expanded (x10) recordings of signals produced by bats were made using a D240x ultrasound detector with a sampling frequency of 307 kHz (Pettersson Electronik AB, Sweden) and stored digitally on an Edirol R-09HR recorder (Roland, USA) using a sampling rate of 44.1 kHz, with 16 bits/sample. Recordings for rhinolophid and hipposiderid bats were obtained from motionless bats held in the hand, whereas recordings for all other species were obtained in flight either from hand-released bats, a flight cage (measuring 10 x 2 x 1.5 m) or a tethered zip-line (Sweczak, 2000). Because habitat structure induces variation in echolocation calls (Schnitzler *et al*., 2003), we acknowledge that this means our sample is biased towards call types that are characteristic of more cluttered environments (i.e. broader bandwidths and shorter durations).

Signal analysis was undertaken using BatSound (vers. 3.31, Pettersson Electronik AB, Sweden). To avoid pseudo-replication, one call per bat was selected for description of call parameters and subsequent analysis. Because two species (*Megaderma spasma* and *Myotis annectans*) were represented by only two individuals, however, two calls were analysed for each of these individuals. Additionally, as only one individual was captured of each of four species (*Hipposideros cineraceus, Kerivoula hardwickii, Tylonycteris pachypus* and *Miniopterus pusillus*), four calls were analysed for each of these individuals.

For each call, five parameters were measured: call duration (duration of a single pulse), inter-pulse interval (IPI, time from the start of one call to the onset of the next), start frequency (frequency value at the start of the call), end frequency (frequency value at the end of the call) and peak frequency (FmaxE, frequency of maximum energy for the whole call). Call duration and IPI (ms) were obtained from oscillograms, FmaxE (kHz) from power spectra, whereas the start frequency (kHz) and end frequency (kHz) were measured from spectrograms using a 512-size Fast Fourier Transformation and a Hanning window. An additional parameter, duty cycle (the amount of time a bat spends calling relative to the amount of time it is silent), was calculated by dividing the call duration by the inter-pulse interval and multiplying by 100 (for a percentage). All measurements were taken from the call harmonic containing the greatest energy. The position of the harmonic containing the most energy and number of harmonics present in each call were also noted for the purposes of describing the echolocation calls produced by each species.

Statistical procedures

To test the efficacy of acoustic data in correctly identifying bat species, a discriminant function analysis was performed. Species represented by a single individual were excluded from the analysis (*H. cineraceus, K. hardwickii, T. pachypus* and *M. pusillus*).

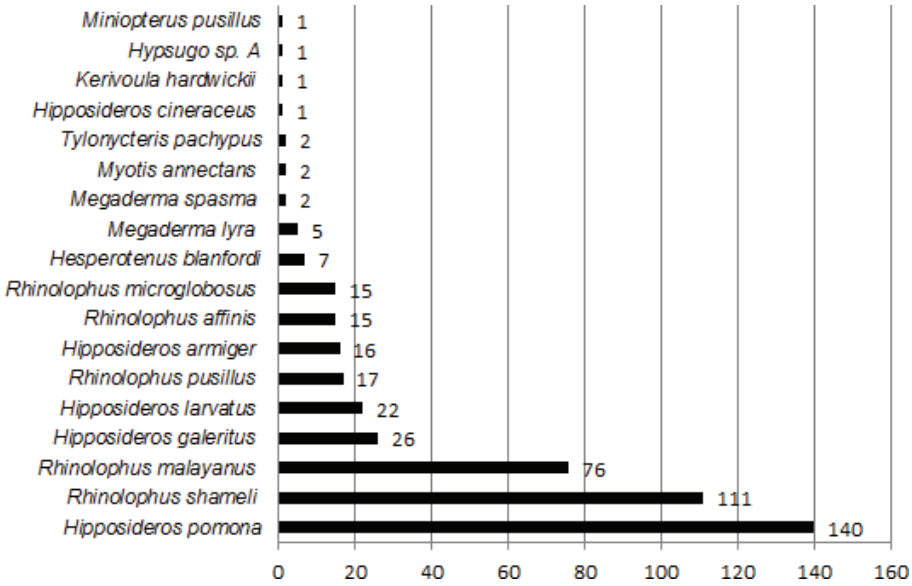

Fig. 2 Relative abundance of echolocating bat species captured in Phnom Kulen National Park, Cambodia. Figures show the total number of individuals captured.

Because examination of covariance matrices using Box's *M* test indicated that these were not homogenous ($F = 13.653$, $P < 0.001$), a quadratic discriminant function analysis was applied. Cross-validation was employed in the analysis. Multivariate analysis of variance (MANOVA) was conducted to examine the significance of the discriminant function analysis models and Wilk's λ values were used to determine the discrimination power of each variable. All tests were performed using MINITAB (vers. 15.0), with the exception of Box's *M* test which was performed using SPSS Statistics (vers. 16.0). In all tests, values of $P < 0.05$ were considered significant.

Results

Species captured

Over the course of the fieldwork, 460 individuals representing 18 echolocating bat species were captured in five families (Megadermatidae: two species; Rhinolophidae: five species; Hipposideridae: five species; Vespertilionidae: five species; Miniopteridae: one species). Relative species abundance was highly uneven with three species representing 71.1% of all captures: *Hipposideros pomona* (140 individuals, 30.4% of captures), *Rhinolophus shameli* (111, 24.1%) and *R. malayanus* (76, 16.5%) (Fig. 2).

One hundred and fifteen individuals (25%) were captured in mist nets, and 345 (75%) were caught in harp traps. Of the 18 echolocating bat species encountered, eight were captured in mist nets and harp traps (*Rhinolophus affinis, R. malayanus, R. pusillus, R. shameli, R. microglobosus, Hipposideros galeritus, H. larvatus* and *H. pomona*), five in mist nets only (*Megaderma lyra, M. spasma, Hipposideros armiger, Hesperoptenus blanfordi* and *Myotis annectans*) and five exclusively in harp traps (*Hipposideros cineraceus, Hypsugo* sp. A., *T. pachypus, K. hardwickii* and *Miniopterus pusillus*).

Description of echolocation calls

Time-expanded recordings of 444 echolocation calls were analysed for all but one of the 18 species captured during the fieldwork. Recordings were not obtained for a single individual designated as *Hypsugo* sp. A, for which the correct specific name has yet to be determined.

The five rhinolophid species in our sample produced calls characterised by a long constant frequency (CF) component which was preceded and terminated by a brief frequency-modulated (FM) component (Table 1, Fig. 3a). The second call harmonic invariably contained the most energy and all five species operated at high duty cycles, with mean values ranging from 73.6 ± 13.9% in *R. malayanus* to 84.2 ± 3% in *R. pusillus*. Peak frequency (FmaxE) values ranged from 69.5 ± 1.7 kHz in *R. shameli* to 112.2 ± 1.3 kHz in *R.*

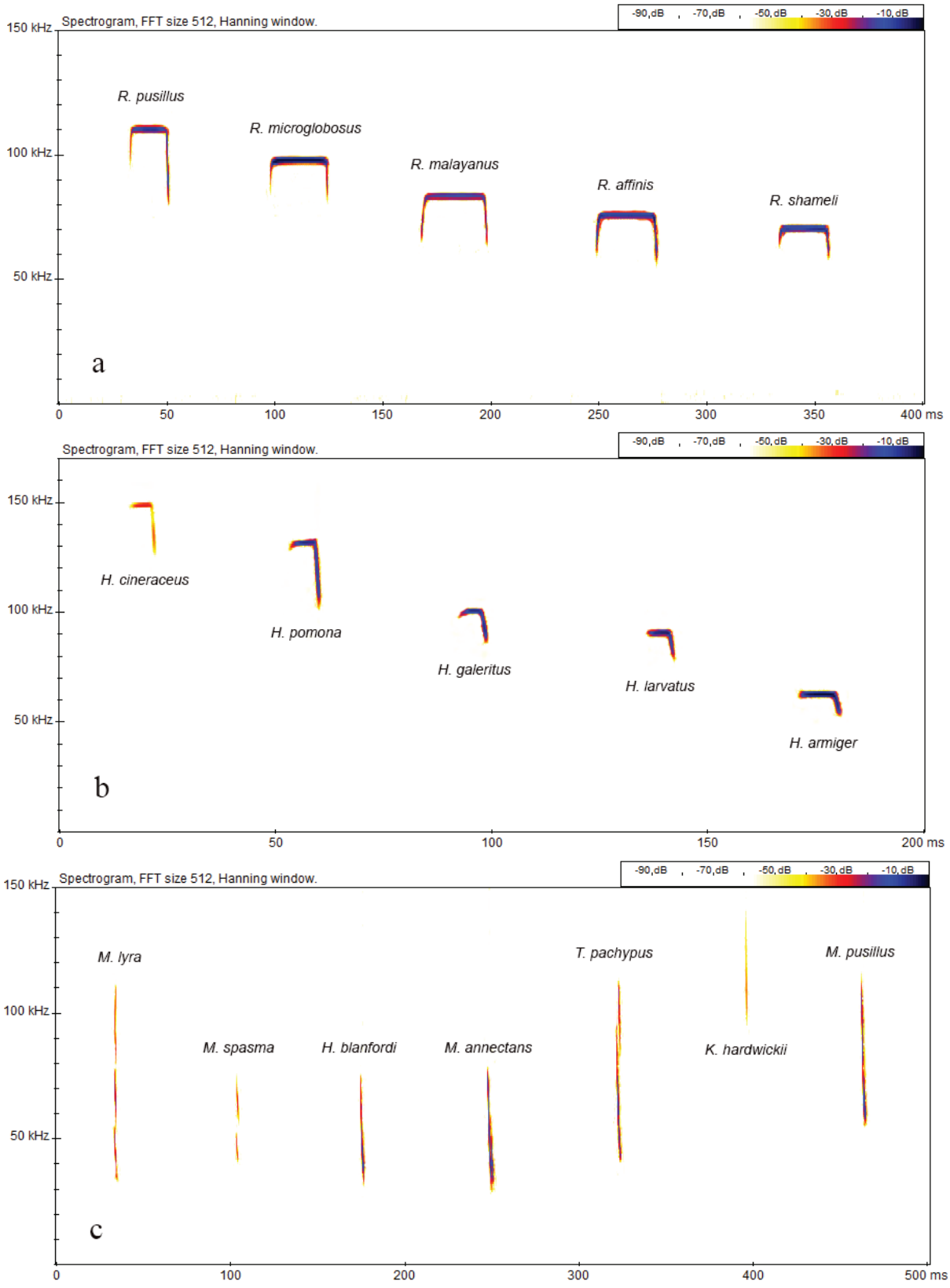

Fig. 3 Echolocation calls of 17 bat species in Phnom Kulen National Park: (a) Rhinolophidae; (b) Hipposideridae; (c) Megadermatidae, Vespertilionidae and Miniopteridae. (Note differences in *x*-axis values between figures).

Table 1 Echolocation call parameters of five rhinolophid and five hipposiderid bat species at Phnom Kulen National Park, Cambodia.

Species	Call Structure	Start Frequency (kHz)	End Frequency (kHz)	Frequency of Maximum Energy (kHz)	Call Duration (ms)	Inter-Pulse Interval (ms)	Duty Cycle (%)	*n*
RHINOLOPHIDAE								
Rhinolophus affinis	FM/CF/FM	70.5 ± 3.1 (66–76)	61.5 ± 2 (60–66)	77.1 ± 0.5 (76.5–78.3)	26.6 ± 5.9 (17.9–36.7)	36.9 ± 14 (20.8–63)	76.2 ± 12.5 (54.1–88.5)	15
Rhinolophus malayanus	FM/CF/FM	76.1 ± 3.9 (71–82)	78 ± 4.8 (62–83)	83 ± 0.7 (81.1–84.7)	27.1 ± 8.8 (14.8–61.8)	40.9 ± 24.4 (19.1–131.4)	73.6 ± 13.9 (26.2–86.4)	61
Rhinolophus pusillus	FM/CF/FM	101.4 ± 4.6 (95–111)	96.8 ± 3.2 (95–105)	112.2 ± 1.3 (108.9–114.1)	22.8 ± 5.3 (14.7–34.1)	27 ± 5.5 (18.2–38.6)	84.2 ± 3 (78.6–89.2)	17
Rhinolophus shameli	FM/CF/FM	64 ± 3.1 (52–69)	61 ± 1.9 (59–69)	69.5 ± 1.7 (65.4–71.8)	31.9 ± 6.6 (15.5–46.4)	45.3 ± 22.1 (18.2–171.7)	76.8 ± 15.1 (22.8–90.9)	105
Rhinolophus microglobosus	FM/CF/FM	94 ± 2.2 (90–97)	93.9 ± 2.1 (90–96)	98.3 ± 0.6 (96.4–98.9)	26.2 ± 7.1 (14.4–39)	34.8 ± 8.5 (21.7–49.4)	74.7 ± 3.4 (66.4–80.1)	15
HIPPOSIDERIDAE								
Hipposideros armiger	CF/FM	62.7 ± 0.7 (62–64)	55.7 ± 1.4 (53–58)	63.9 ± 0.8 (61.4–65)	10.4 ± 1.7 (8–14.4)	41.3 ± 14.8 (26.8–80.1)	26.7 ± 5.5 (15.7–35.8)	16
*Hipposideros cineraceus**	CF/FM	149 ± 0.8 (148–150)	129.3 ± 1.3 (128–131)	150 ± 0.8 (149.2–150.6)	5.5 ± 0.4 (5–5.9)	15.6 ± 2.8 (12.1–18.7)	36.2 ± 4.8 (31.6–41.3)	4
Hipposideros galeritus	CF/FM	99.5 ± 1.4 (97–102)	90.1 ± 1.5 (87–92)	100.7 ± 1 (98.5–102.5)	5.9 ± 1 (3.9–8.7)	23.3 ± 6 (10.9–37.8)	26.5 ± 7.4 (17.8–53.2)	23
Hipposideros larvatus	CF/FM	91.5 ± 0.8 (90–93)	81.5 ± 1.5 (80–87)	92.3 ± 0.8 (90.8–93.5)	6.6 ± 1.2 (5.1–9)	22.6 ± 9.2 (15.3–52.8)	31.5 ± 7.9 (13.6–46.2)	21
Hipposideros pomona	CF/FM	134 ± 1.8 (128–139)	111.3 ± 2.9 (105–111.3)	134.8 ± 1.8 (128.3–139.7)	5.1 ± 0.7 (3.7–7.5)	12.2 ± 7.6 (7.1–93.6)	44.9 ± 7.5 (7.1–60)	135

* One call per individual bat was analysed except for *H. cineraceus*, for which four calls from the same individual were analysed. CF = constant frequency; FM = frequency-modulated. Values are given as mean ± SD (min–max).

pusillus, whereas call duration ranged from 22.8 ± 5.3 ms in *R. pusillus* to 31.9 ± 6.6 ms in *R. shameli*. FmaxE values did not overlap between species, indicating this call parameter will be helpful for the field identification of all of the rhinolophid species in our sample from Phnom Kulen.

The five hipposiderids that were analysed produced calls beginning with a relatively long and almost CF component which terminated in a comparatively brief and downward FM component (Table 1, Fig. 3b). This structure facilitates unequivocal separation of hipposiderid calls from all other bat families in Phnom Kulen. All species produced calls with the greatest energy in the second harmonic and operated at lower duty cycles (mean values ranging from 26.5 ± 7.4% in *Hipposideros galeritus* to 44.9 ± 7.5% in *H. pomona*) than rhinolophids due to their shorter and non-overlapping call durations. Mean values for the latter ranged from 5.1 ± 0.7 ms in *H. pomona* to 10.4 ± 1.7 ms in *H. armiger*, while mean FmaxE values ranged from 63.9 ± 0.8 kHz in *H. armiger* to 150 ± 0.8 kHz in *H. cineraceus*. Like the rhinolophids, FmaxE values did not overlap between species indicating this call parameter will also aid field identification of all hipposiderids in our sample from Phnom Kulen.

The two megadermatids in our sample produced multi-harmonic FM calls (Table 2, Fig. 3c). *Megaderma lyra* emitted signals with a mean FmaxE of 64.7 ± 2.6 kHz, a mean call duration of 2.4 ± 0.8 ms and the third harmonic contained the greatest energy. *Megaderma spasma* produced calls of similar frequency with a mean FmaxE of 65.4 ± 3.1 kHz (third harmonic), but mean call durations were somewhat shorter at 1.1 ± 0.2 ms and the first harmonic appeared to be suppressed.

The four vespertilionids that were analysed produced steep, downward FM calls dominated by the fundamental harmonic (Table 2, Fig. 3c). All four species produced relatively brief calls—mean durations ranging from 0.5 ± 01 ms in *K. hardwickii* to 2.4 ± 0.3 ms in *Myotis annectans*. Mean duty cycles were generally higher than those of megadermatids

Table 2 Echolocation call parameters of two megadermatid, four vespertilionid and one miniopterid bat species at Phnom Kulen National Park, Cambodia.

Species	Call Structure	Start Frequency (kHz)	End Frequency (kHz)	Frequency of Maximum Energy (kHz)	Call Duration (ms)	Inter-Pulse Interval (ms)	Duty Cycle (%)	*n*
MEGADERMATIDAE								
Megaderma lyra	FM	72.4 ± 4.1 (66–76)	57 ± 4.1 (53–62)	64.7 ± 2.6 (61.6–67.5)	2.4 ± 0.8 (1.4–3.2)	93.6 ± 38.3 (38.9–134.1)	2.7 ± 0.7 (1.8–3.6)	5
*Megaderma spasma**	FM	70.8 ± 4.3 (65–74)	62.5 ± 2.1 (60–65)	65.4 ± 3.1 (61.6–69.3)	1.1 ± 0.2 (1–1.3)	68.3 ± 41.9 (25.9–104.4)	2.3 ± 1.5 (1–3.9)	4
VESPERTILIONIDAE								
Hesperoptenus blanfordi	FM	58 ± 10.8 (45–72)	39.3 ± 7 (35–54)	46.5 ± 6.6 (41.9–61.1)	1.5 ± 0.4 (1.2–2)	40 ± 25.6 (14.3–92.3)	4.7 ± 1.9 (2–7.7)	7
*Myotis annectans**	FM	50.8 ± 0.5 (50–51)	38 ± 1.2 (37–39)	39.8 ± 0.7 (39.2–40.8)	2.4 ± 0.3 (2.1–2.8)	54.4 ± 21.1 (31.1–80)	5.2 ± 2.8 (2.9–9)	4
*Tylonycteris pachypus***	FM	68.5 ± 3 (65–71)	46.3 ± 1.5 (45–48)	64.7 ± 1.2 (63.9–66.5)	1.8 ± 0.3 (1.5–2.1)	25 ± 12.6 (14.1–39.5)	8.7 ± 4.8 (3.8–14.1)	4
*Kerivoula hardwickii***	FM	114.8 ± 10.9 (104–126)	101.3 ± 1.5 (99–102)	103.3 ± 2.2 (100.7–106)	0.5 ± 0.1 (0.4–0.6)	15.9 ± 2 (13.8–18)	3 ± 0.4 (2.7–3.5)	4
MINIOPTERIDAE								
*Miniopterus pusillus***	FM	73.5 ± 8.6 (63–84)	59.8 ± 0.5 (58–60)	60.8 ± 0.6 (60.2–61.6)	3.6 ± 0.3 (3.4–3.9)	48 ± 7 (39.7–54.3)	7.7 ± 1.2 (6.3–8.7)	4

One call per bat was analysed except for species marked * for which two calls per individual were measured, and ** for which four calls per individual were measured. CF = constant frequency; FM = frequency-modulated. Values are given as mean ± SD (min–max).

Table 3 Cross-validated classification matrix for species emitting CF calls (genera *Hipposideros* and *Rhinolophus*).

	True Groups								
Classified as	*Hipposideros armiger*	*Hipposideros galeritus*	*Hipposideros larvatus*	*Hipposideros pomona*	*Rhinolophus affinis*	*Rhinolophus malayanus*	*Rhinolophus pusillus*	*Rhinolophus shameli*	*Rhinolophus microglobosus*
Hipposideros armiger	16	0	0	0	0	0	0	0	0
Hipposideros galeritus	0	23	0	0	0	0	0	0	0
Hipposideros larvatus	0	0	21	0	0	0	0	0	0
Hipposideros pomona	0	0	0	135	0	0	0	0	0
Rhinolophus affinis	0	0	0	0	15	0	0	0	0
Rhinolophus malayanus	0	0	0	0	0	61	0	0	0
Rhinolophus pusillus	0	0	0	0	0	0	17	0	0
Rhinolophus shameli	0	0	0	0	0	0	0	105	0
Rhinolophus microglobosus	0	0	0	0	0	0	0	0	15
Total *n*	16	23	21	135	15	61	17	105	15
no. correct	16	23	21	135	15	61	17	105	15
% correct	100	100	100	100	100	100	100	100	100

The discriminant function analysis model relied on two parameters (Duration and FmaxE) and provided an overall correct classification rate of 100% when cross-validated.

and mean FmaxE values ranged from 39.8 ± 0.7 kHz in *M. annectans* to 103.3 ± 2.2 kHz in *K. hardwickii.* The only miniopterid in our sample, *Miniopterus pusillus*, produced steep, downward FM signals similar to vespertilionids, but of longer mean call duration (3.6 ± 0.3 ms) and a mean FmaxE of 60.8 ± 0.6 kHz.

Discriminant function analysis

Thirteen bat species were assessed in the analysis. As these could be unequivocally separated into two groups by their call structures, quadratic discriminant analysis was undertaken for (i) species whose calls contained a CF portion terminating in an FM portion (five rhinolophids and four hipposiderids) ("CF group"); and (ii) species whose calls comprised an FM signal (two megadermatids and two vespertilionids) ("FM group").

Quadratic discriminant function analysis for the CF group resulted in a 100% correct classification rate (408 calls correctly classified) which remained unchanged when cross-validated (Table 3). The best model relied upon two parameters (call duration and FmaxE), which a MANOVA showed was significant (Wilk's λ = 0.00102, F = 1510.276, P < 0.001). Wilk's λ values indicated that the discrimination power of the two variables in decreasing order was: FmaxE (0.00271) and call duration (0.16016).

Table 4 Cross-validated classification matrix for species emitting FM calls (genera *Megaderma*, *Hesperoptenus* and *Myotis*).

	True Groups			
Classified as	*Megaderma lyra*	*Megaderma spasma*	*Hesperoptenus blanfordi*	*Myotis annectans*
Megaderma lyra	3	1	0	0
Megaderma spasma	2	3	1	0
Hesperoptenus blanfordi	0	0	6	2
Myotis annectans	0	0	0	2
Total *n*	5	4	7	4
no. correct	3	3	6	2
% correct	60.0	75.0	85.7	50.0

The discriminant function analysis model relied on two parameters (FmaxE, IPI) and provided an overall correct classification rate of 70.0% when cross-validated.

Despite the small sample sizes for species in the FM group, the best model (relying on two call parameters: FmaxE and IPI) produced a 90% correct classification rate (18 calls correctly classified out of 20) and a 70% correct classification rate when cross-validated (14 calls correctly classified) (Table 4). MANOVA demonstrated that the model was significant (Wilk's λ = 0.08927, F = 11.735, P < 0.001) and Wilk's λ values indicated that the discrimination power of the two variables in decreasing order was: FmaxE (0.1224) and IPI (0.65160).

Discussion

Ours is the first study to describe the echolocation calls produced by a Cambodian bat assemblage and in achieving a correct, cross-validated classification rate of 85% overall, our results indicate that correct acoustic identification of in-country bat species is feasible using the call parameters we employed.

The call parameters we recorded for each species are generally consistent with those of other studies in the region (Soisook *et al*., 2008; Furey *et al*., 2009; Douangboubpha *et al*., 2010; Hughes *et al*., 2010, 2011; Kingsada *et al*., 2011; Ith *et al*., 2011b), although because sample sizes for megadermatid, vespertilionid and miniopterid species were small (and for reasons stated in the methods), it is highly unlikely these encompass the full range of variation in calls produced by these taxa. For instance, because *Kerivoula* spp. are known to produce signals with very high starting frequencies (Kingston *et al*., 1999; Schmieder *et al*., 2010), these were likely missed in our recordings due to insufficient sampling frequencies, resulting in lower starting frequencies and shorter call durations. Notwithstanding this, the rate of correct classification we obtained in discriminant function analysis is comparable to similar studies of bats around the world. For instance, MacSwiney *et al*. (2008) achieved a correct classification rate of 84% for 26 species in Mexico; Russo & Jones (2002) a rate of 82% for 22 species in Italy; and Kofoky *et al*. (2009) a rate of 82% for 15 species in Madagascar. Our results thus support previous suggestions (Furey *et al*., 2009) that acoustic identification of free-flying bats is an equally achievable goal in Southeast Asia.

Significant additional research will be required to realise this goal, however. As intra-specific variation occurs in echolocation calls due to geographical location (Thomas *et al*., 1987), reference recordings from every site under investigation will be required to reliably identify species whose call parameters

may overlap with those of others in certain parts of their range. Second, as habitat structure also induces variation in echolocation calls (Schnitzler *et al*., 2003), recordings from a range of structural environments will be required to elucidate the full repertoire of calls produced by different species. This will require significant field effort to obtain sufficient recordings for less abundant (or simply rarely captured) taxa, as demonstrated by the highly uneven relative species abundances encountered in the present study.

Because acoustic methods are unlikely to improve upon results provided by harp traps for bat species that echolocate at very low intensities (e.g. species within the Murininae and Kerivoulinae and *Coelops frithii*) (Furey *et al*., 2009), this approach is perhaps best regarded as an important complement to, rather than a replacement of, traditional capture methods for inventorying echolocating bats in Southeast Asia. As the taxonomy of many Southeast Asian bats remains uncertain (Francis *et al*., 2010), the need for live-trapping and collecting voucher samples to ensure correct assignment of names and recognition of species limits will inevitably also continue. We nonetheless recommend further studies to facilitate development of acoustic sampling as a tool for improving understanding and conservation of Cambodian bats.

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Annex 1

RHINOLOPHIDAE—*Rhinolophus affinis*: CBC00927, male, in spirit, skull removed, collected on 25 April 2010, 13°41.028N, 104°01.366E, 82 metres above sea level (m a.s.l.); CBC00942, CBC00943, males, in spirit, skulls removed, collected on 23 June 2010, 13°41.409N, 104°00.733E, 177 m a.s.l.; CBC00947, CBC00948, CBC00949, males, in spirit, skulls removed, collected on 24 July 2010, 13°40.301N, 104°01.510E, 72 m a.s.l. (described by Kingsada *et al*., 2011). *Rhinolophus malayanus*: CBC00904, male, in spirit, skull removed, collected on 21 April 2010, 12°46.887N, 103°27.806E, 205 m a.s.l.; CBC00921, male, in spirit, skull removed, collected on 22 April 2010, 13°40.855N, 104°01.244E, 72 m a.s.l. *Rhinolophus pusillus*: CBC00933, male, in spirit, skull removed, collected on 19 May 2010, 13°41.295N, 104°00.739E, 215 m a.s.l.; CBC00935, female, in spirit, skull removed, collected on 20 May 2010, 13°41.189N, 104°00.642E, 182 m a.s.l. *Rhinolophus shameli*: CBC00905, CBC00906, females, in spirit, skulls removed, collected on 21 April 2010, 12°46.887N, 103°27.806E, 205 m a.s.l.; CBC00926, female, in spirit, skull removed, collected on 25 April 2010, 13°41.028N, 104°01.366E, 82 m a.s.l.; CBC00928, female, in spirit, skull removed, collected on 18 May 2010, 13°41.340N, 104°00.668E, 171 m a.s.l. *Rhinolophus microglobosus*: CBC00901, male, in spirit, skull removed, collected on 21 April 2010, 12°46.887N, 103°27.806E, 205 m a.s.l.; CBC00930, female, in spirit, skull removed, collected on 18 May 2010, 13°41.340N, 104°00.668E, 171 m a.s.l.; CBC00936, female, in spirit, skull removed, collected on 20 May 2010, 13°41.189N, 104°00.642E, 182 m a.s.l.

HIPPOSIDERIDAE—*Hipposideros armiger*: CBC00923, CBC00924, male and female, in spirit, skulls removed, collected on 24 April 2010, 13°40.944N, 104°01.134E, 97 m a.s.l.; CBC00938, male, in spirit, skull removed, collected on 23 May 2010, 13°40.598N, 104°01.506E, 66 m a.s.l.; CBC00941, female, in spirit, skull removed, collected on 22 June 2010, 13°41.495N, 104°00.647E, 204 m a.s.l. *Hipposideros cineraceus*: CBC00944, female, in spirit, skull removed, collected on 26 June 2010, 13°40.816N, 104°00.973E, 183, m a.s.l. *Hipposideros galeritus*: CBC00898, CBC00899, CBC00900, two females and one male, in spirit, skulls removed, collected on 21 April 2010, 12°46.887N, 103°27.806E, 205 m a.s.l.; CBC00931, male, in spirit, skull removed, collected on 18 May 2010, 13°41.340N, 104°00.668E, 171 m a.s.l.; CBC00932, male, in spirit, skull removed, collected on 19 May 2010, 13°41.295N, 104°00.739E, 215 m a.s.l.; CBC00950, male, in spirit, skull removed, collected on 25 July 2010, 13°40.092N, 104°01.399E, 68 m a.s.l. *Hipposideros larvatus*: CBC00925, female, in spirit, skull removed, collected on 27 April 2010, 13°40.949N, 104°01.416E, 80 m a.s.l.; CBC00929, female, in spirit, skull removed, collected on 18 May 2010, 13°41.340N, 104°00.668E, 171 m a.s.l. *Hipposideros pomona*: CBC00902, male, in spirit, skull removed, collected on 21 April 2010, 12°46.887N, 103°27.806E, 205 m a.s.l.; CBC00903, female, in spirit, skull removed, collected on 24 April 2010, 13°40.944N, 104°01.134E, 97 m a.s.l.; CBC00934, female, in spirit, skull removed, collected on 20 May 2010, 13°41.189N, 104°00.642E, 182 m a.s.l.; CBC00939, CBC00940, male and female, in spirit, skulls removed, collected on 20 June 2010, 13°40.796N, 104°01.593E, 65 m a.s.l.

MEGADERMATIDAE—*Megaderma lyra*: CBC00919, female, in spirit, skull removed, collected on 21 April 2010, 12°46.887N, 103°27.806E, 205 m a.s.l.; CBC00920, female, in spirit, skull removed, collected on 24 April 2010, 13°40.944N, 104°01.134E, 97 m a.s.l. *Megaderma spasma*: CBC00945, female, in spirit, skull removed, collected on 20 July 2010, 13°41.339N, 104°00.970E, 188 m a.s.l.

VESPERTILIONIDAE—*Hesperoptenus blanfordi*: CBC00907, CBC00911, CBC00912, CBC00913, CBC00914, CBC00915, CBC00916, four females and three males, in spirit, skulls removed, collected on 21 April 2010, 12°46.887N, 103°27.806E, 205 m a.s.l. *Hypsugo* sp. A: CBC00917, male, in spirit, skull and baculum removed, collected on 21 April 2010, 12°46.887N, 103°27.806E, 205 m a.s.l. *Myotis annectans*: CBC00909, CBC00918, females, in spirit, skulls removed, collected on 21 April 2010, 12°46.887N, 103°27.806E, 205 m a.s.l.; CBC00937, female, in spirit, skull removed, collected on 23 May 2010, 13°40.598N, 104°01.506E, 66 m a.s.l. *Tylonycteris pachypus*: CBC00908, CBC00910, females, in spirit, skulls removed, collected on 21 April 2010, 12°46.887N, 103°27.806E, 205 m a.s.l. *Kerivoula hardwickii*: CBC00946, female, in spirit, skull removed, collected on 21 July 2010, 13°41.574N, 104°00.633E, 206 m a.s.l.

MINIOPTERIDAE—*Miniopterus pusillus*: CBC00951, male, in spirit, skull removed, collected on 25 July 2010, 13°39.810N, 104°01.862E, 68 m.a.s.l (described by Furey *et al*., 2012).