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Article

# Reproductive Ecology of Wrinkle-Lipped Free-Tailed Bats *Chaerephon plicatus* (Buchanan, 1800) in Relation to Guano Production in Cambodia

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**Abstract:** Wildlife populations in Southeast Asia are subject to increasing pressure from climate change, habitat loss and human disturbance. Cave-roosting bats are particularly vulnerable to all three factors. Because of the ecological services they provide, it is important to assess specific vulnerabilities to inform their conservation management. We evaluated the reproductive phenology and body condition of *Chaerephon plicatus* for 14 months in 2015–2016 and quantified guano harvesting at the largest colony in Cambodia in 2011–2016. As in Thailand and Myanmar, two annual breeding cycles were recorded, characterized as continuous bimodal polyoestry, with parturition primarily occurring in April and October. Significant declines occurred in body condition between the late wet season and the late dry season, suggesting that bats experience increasing energetic stress as the dry season progresses. Annual guano harvests increased over the study period but could not be used as a proxy for monitoring population size due to the loss of unknown amounts during the wet season and unquantified movements of bats between *C. plicatus* colonies in the region. We recommend studies to determine the scale and drivers of such movements and creation of sustainable guano harvesting and population monitoring initiatives to ensure the conservation of *C. plicatus* colonies in Cambodia.

**Keywords:** cave bats; reproduction; body condition; guano harvesting; seasonal tropics

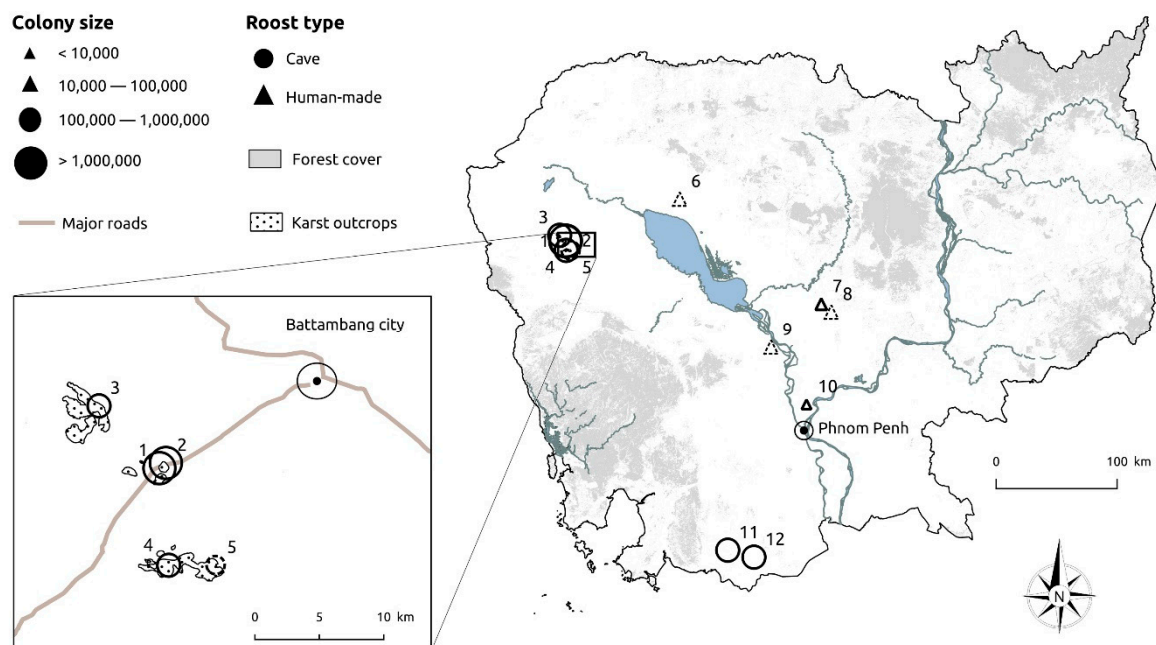
## 1. Introduction

Wildlife populations are subject to increasing pressure from a range of threatening processes worldwide, including climate change, habitat loss and human disturbance. This is particularly true in Southeast Asia, a region supporting >25% of global bat diversity [1], which has one of the highest deforestation rates in the tropics [2], and where human populations doubled in under four decades to reach 593 million people in 2010 [3]. As a consequence, information regarding the specific vulnerabilities of regionally diverse and threatened taxa such as cave-roosting bats [4] is of considerable interest and can be used to guide efforts to protect and sustainably manage their populations.

The wrinkle-lipped free-tailed bat *Chaerephon plicatus* (synonym: *Tadarida plicata*) (Molossidae) is a widely-distributed insectivorous species occurring from India and Sri Lanka through mainland

Southeast Asia and southern China, to the Philippines and Indonesia [5]. The taxon is perhaps best known for aggregating in colonies of up to several million bats in caves, although it also roosts in rock crevices and buildings [6] and is presumed to have a nightly foraging radius of 25 km (equating to a foraging catchment of nearly 2000 km<sup>2</sup>) [7]. Large aggregations are characteristic of molossid bats in both New and Old World caves and the evening emergence of such colonies attracts significant numbers of tourists every year [4]. They are also functionally important due to the substantial services they provide in the suppression of arthropod pests. For instance, the pest predation services provided by free-tailed bats (*Tadarida brasiliensis*) in protecting cotton yields in the southern US were valued at 741,000 USD annually [8], whereas in Thailand, similar biological control provided by *C. plicatus* in protecting rice harvests was valued at 1.2 million USD each year [7].

The destruction of a roost of  $\approx 2$  million *C. plicatus* at the National Museum in the Cambodian capital of Phnom Penh in the early 2000s [9,10] led to the documentation of 12 large colonies of the species throughout the country (Table 1, Figure 1). These include seven colonies inhabiting limestone caves in north-western and southern Cambodia which may represent  $\approx 97\%$  (6.37 million bats) of the national population [11]. All seven colonies have been evaluated as nationally significant sites for bat conservation [12], whereas the current status of the five smaller colonies identified at scattered anthropogenic roost sites in central Cambodia [11] remains unknown. The large quantities of guano produced by the seven cave colonies are harvested for plant fertilizer by local communities and should conservative estimates of the value of ecosystem services provided by these bats in Thailand [7] hold true for Cambodia, these would prevent the loss of >2300 tons of rice or daily rice meals for >20,800 people each year. Given the functional and economic importance of this service, the aim of our study was to determine requirements for sustainable management of the species in Cambodia.



**Table 1.** Summary characteristics of major *Chaerephon plicatus* colonies in Cambodia.

#	Site Name (Hill, Province)	Latitude, Longitude	Roost Type/Status	Colony Size <sup>1</sup>
1	Ta Rumm #1 <sup>2</sup> (Sampeu hill, Battambang)	13.022 N, 103.095 E	Cave/Permanent	1,800,000
2	La Ang Pracheu <sup>2</sup> (Sampeu hill, Battambang)	13.026 N, 103.099 E	Cave/Permanent	1,000,000
3	La Ang Prakiu #2 <sup>2</sup> (Takriem hill, Battambang)	13.066 N, 103.051 E	Cave/Permanent	800,000
4	La Ang Pracheav <sup>2</sup> (Reichiatra hill, Battambang)	12.954 N, 103.102 E	Cave/Permanent	600,000
5	La Ang Sankeur <sup>2</sup> (Sankeur hill, Battambang)	12.955 N, 103.135 E	Cave/Seasonal	900,000
6	Ba Kang <sup>3</sup> (Siem Reap)	13.341 N, 103.966 E <sup>4</sup>	Pagoda/Seasonal	30,000
7	Kampong Thma <sup>3</sup> (Kampong Thom)	12.560 N, 105.047 E <sup>4</sup>	Pagoda/Permanent	80,000
8	Tang Krosang <sup>3</sup> (Kampong Thom)	12.501 N, 105.124 E <sup>4</sup>	Pagoda/Seasonal	70,000
9	Yeay Tep <sup>3</sup> (Kampong Chhnang)	12.233 N, 104.665 E <sup>4</sup>	Pagoda/Seasonal	10,000
10	Unnamed site <sup>3</sup> (Kandal)	11.804 N, 104.935 E <sup>4</sup>	Stupa/Permanent	4000
11	Loang <sup>2</sup> (Loang hill, Kampot)	10.714 N, 104.339 E	Cave/Permanent	900,000
12	Vihear Luong <sup>2</sup> (Koun Sat hill, Kampot)	10.662 N, 104.538 E	Cave/Permanent	370,000

<sup>1</sup> Based on Reference [11]. Site names follow <sup>2</sup> Reference [12], or <sup>3</sup> Reference [11]. <sup>4</sup> Approximate location.

Because cave-roosting bats are vulnerable to human disturbance during critical reproductive periods (late pregnancy, lactation and weaning) [4], documentation of these periods has clear conservation relevance [13]. Although a wide variety of reproductive patterns have been described for molossid bats [14], the similar climate and monsoon seasons of Thailand and Myanmar [15] led us predict that reproductive phenology of *C. plicatus* in Cambodia would match patterns documented in these countries [16–18]. Due to the influence of climate and specifically rainfall on insect biomass and hence food availability for insectivorous bats [14,19,20], we further hypothesized that differences in the latter between the dry and wet seasons in Cambodia would be reflected in seasonal differences in body condition and consequently permit identification of periods when the species may be subject to energetic stress. Lastly, we quantified guano harvests at the largest known and most closely protected *C. plicatus* colony in Cambodia to assess their utility for future monitoring efforts.

## 2. Materials and Methods

### 2.1. Study Sites

Our field work was undertaken in 2015–2016 at Ta Rumm #1 cave, Sampeu hill and La Ang Pracheav cave, Reichiatra hill in Battambang Province, north-western Cambodia (Table 1, Figure 1). Though Ta Rumm #1 cave was the study focus, La Ang Pracheav cave was sampled in lieu of this on three occasions in response to the wishes of its concessionaire. Both are consequently described for indicative purposes below. Because the two caves are situated  $\approx 8$  km apart, it was assumed their *C. plicatus* colonies would exhibit the same climate-driven patterns in reproductive phenology and seasonal body condition [14,19,20].

Ta Rumm #1 cave is located midway up the western face of Sampeu hill (area: 0.44 km<sup>2</sup>), a heavily frequented tourist and pilgrimage destination (Site #1: Table 1, Figure 1). Much of the hill is forested, although several Buddhist temples and cave shrines occupy the summit area which is accessible by road. Caves at Sampeu were mapped by [21], including several that served as execution grounds during the Khmer Rouge era. The landscape surrounding the hill is dominated by seasonal (rain fed) rice cultivation, although this is increasingly irrigated and small areas of coconut, banana and other fruit are grown around the base of the hill. Ta Rumm #1 cave has one lower and one upper entrance which are both vertical, and although speleologists have yet to map its interior due to high levels of ammonia [21], these lead to separate roosts within the cave which have both been harvested for guano at least once and as many as four times a month by the same concessionaire since 1995 [22]. Though some *C. plicatus* colonies in Cambodia have been harvested for longer, harvest records are lacking. Each evening, bats departing from the upper cave entrance join those emerging from the lower entrance to form a single stream of departing bats and 1.8 million *C. plicatus* were estimated to occupy the site [11]. Our sampling was conducted in front of the lower cave entrance.

Located  $\approx 8$  km to the south, La Ang Pracheav cave is situated on the upper-northern face of Reichiatra hill (local name, Phnom Romseisak) (Site #4: Table 1, Figure 1). Reichiatra hill forms the western portion of a contiguous series of hills (area: 5.98 km<sup>2</sup>) orientated east-west, much of which are forested and managed locally as a community forest. Like Sampeu hill, the surrounding landscape is dominated by seasonal (rain fed) rice cultivation, although maize is also grown locally. La Ang Pracheav cave is 135 m in length and has a lower and upper entrance, plus two additional ceiling apertures [21]. The lower entrance is horizontal and leads via a level passage to a large chamber at the rear of the cave which was estimated to house 600,000 *C. plicatus* [11]. Our sampling was undertaken at the upper cave entrance, which is effectively vertical in opening directly onto the upper portion of the roost chamber. Following release of the cave from military control in 2014, guano harvesting at La Ang Pracheav has been managed by the board of the community forest [23]. Prior to 2011, the bat colony occupied the cave for three months (July–September) each year but has been in permanent residence since July 2016 [23].

As similar in most seasonal areas in continental Southeast Asia, the climate of north-western Cambodia is dominated by the southwest monsoon which results in heavy rainfall from May to October, and subsequently by the northeast monsoon from November to April when rainfall is scant [15]. Battambang Province has an average annual rainfall of 1312 mm (1982–2013, Battambang Meteorological Station: 13.08° N, 103.21° E). Temperature varies relatively little during the year with mean monthly values for minimum temperatures ranging from 19.3–25.1 °C (in January and May respectively) to maximum temperatures of 30.0–36.1 °C (December and April respectively) (ibid). Approximately 82% of annual rainfall occurs during the wet season and because  $\approx 49\%$  falls in August–October, this is sometimes referred to as the ‘main wet season’.

## 2.2. Data Collection

Two consecutive nights of live-trapping were conducted at one study cave each month from April 2015 to May 2016 inclusive. Sampling occurred during the last ten days of each month, and 11 months of sampling were conducted at Ta Rumm #1 cave, with the remaining three months undertaken at La Ang Pracheav cave (July 2015, April and May 2016).

We captured and handled bats in the field in accordance with guidelines approved by the American Society of Mammalogists [24], in addition to the requirements of the statutory study permission provided by the national authority responsible for wildlife research, the Forestry Administration of the Cambodian Ministry of Agriculture, Forest and Fisheries. As an animal ethics committee did not exist at the time in Cambodia, all study aspects were overseen by the Forestry Administration who participated in the field investigations.

A single mist net (3 × 3 m,  $\approx 100$  denier, 2 ply) was employed in a mobile fashion to sample bats emerging from the entrance of the caves on each occasion. This was held horizontally underneath the stream of emerging bats and shifted to a vertical position for 3–5 s before being returned to horizontal and immediately moved to adjacent ground where the captured bats were extracted and placed in individual cotton drawstring bags by 2–3 surveyors. This procedure began at sunset and was repeated 3–4 times until  $\approx 50$  bats were captured each night. All bats caught were measured for forearm length using dial calipers [6], weighted to the nearest 0.5 g with spring scales (mature males only), identified in-situ using reference keys [25,26] and released unharmed at their capture site the same night.

Because guano harvesting records prior to 2011 for Ta Rumm #1 cave have been lost [22], harvest records were obtained for January 2011 to February 2017 inclusive. Climate data for 2015–2016 were obtained from the Battambang Department of Water Resources and Meteorology, which is located approximately 16 km from the study caves.

## 2.3. Reproductive Assessment

We examined all bats to determine their sex, age and reproductive status [27,28]. We classified volant individuals lacking fully ossified and fused metacarpal-phalangeal epiphyses as juveniles [29].

Genitalia were examined to determine the status of non-juvenile males and these were classified as reproductively immature if they lacked enlarged testes and/or distended caudae epididymides, or mature if these were enlarged or distended [28].

Non-juvenile females were classified as nulliparous or parous, and, reproductively inactive, pregnant, and/or lactating. This was determined by examining the development and morphology of mammary glands and thoracic (axillary) nipples. Pregnancy was assessed by abdominal palpation to determine the presence of fetuses. Lactation was confirmed through the extrusion of milk following gentle palpation of the mammary glands and nipples.

#### 2.4. Analysis

Monthly climate data (rainfall, mean daily minimum and maximum temperatures) for the study period (2015–2016) were contrasted with long term averages (1982–2013). Live-weight served as a proxy for the body condition of bats using the scaled mass index (SMI) [30]. Analyses were confined to reproductively mature male bats to eliminate the confounding effects of reproduction (pregnancy, lactation) and ontogeny (development of juveniles and immature bats) on body weight. Harvesting records for Ta Rumm #1 cave were used to quantify temporal variation in guano production at the site. This is harvested in sacks with a weight of  $\approx 10$  kg and a sale value of 25,000 Riel (=6.25 USD) and all guano is reportedly collected on each harvesting occasion, though this could not be confirmed because of safety concerns associated with accessing the cave due to high levels of ammonia present.

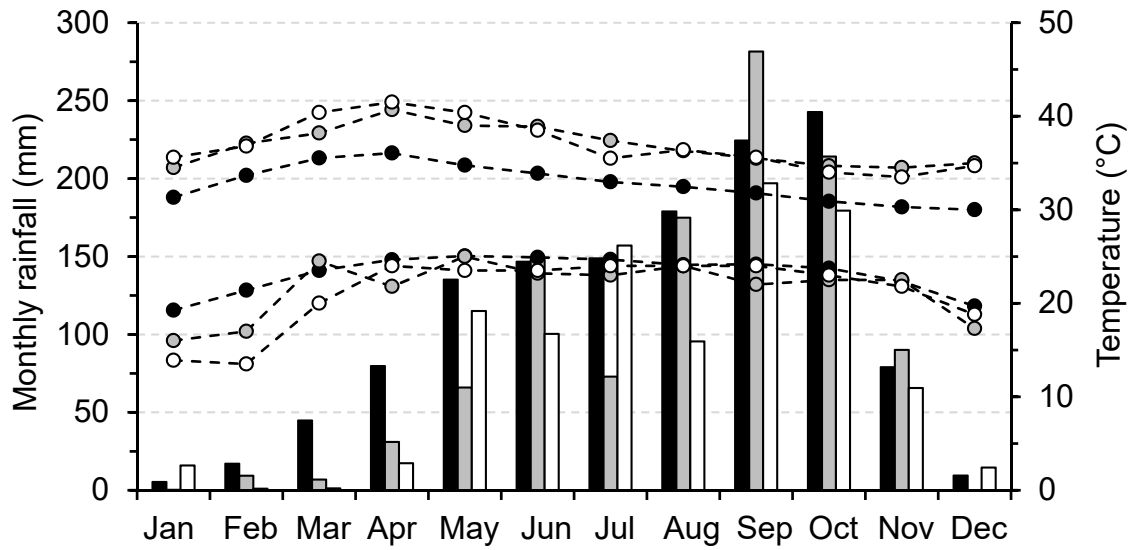
Because monthly percentages of lactating females were not normally distributed, Spearman rank order correlation ( $r_s$ ) was employed to determine the relationship between these values and mean monthly rainfall (1982–2013). Monthly variations in raw SMI values were assessed using a one-way ANOVA test, as these were normally distributed with homogenous variances. Spearman-rank order correlation was also used to assess relationships between mean monthly SMI and monthly rainfall values for August 2015 to May 2016, as the latter were not normally distributed. An independent  $t$ -test was used to compare raw SMI values between the late wet season (August–October 2015) and the late dry season (February–April 2016).

Because data were normal and variances homogenous, a one-way ANOVA test was used to assess variation in total monthly guano harvests between January 2011 and February 2017. Pearson's product-moment correlation ( $r$ ) was used to explore relationships between mean monthly guano harvests for the same period and mean monthly rainfall (1982–2013), as both data were also normal. An independent  $t$ -test was employed to compare total monthly guano harvests between the dry (November–April) and wet seasons (May–October) from January 2011 and December 2016. All tests were carried out using SPSS vers. 23 or Minitab vers. 17.1.0 and the threshold for significance was set at  $p < 0.05$ .

### 3. Results

#### 3.1. Climatic Variation

The wet season in 2015 and 2016 was typical for Battambang and occurred from May to October, although less rain fell in both years compared to long term averages for the province (1095 and 960 mm respectively vs. 1312 mm) (Figure 2, Table S1). Mean daily maximum temperatures each month were also consistently greater than long term averages, particularly in March–May 2016 when they reached 40.4–41.5 °C (4.9–5.6 °C higher). Apart from the first two months of each study year, average daily minimum temperatures each month were more similar to long term means, although they were slightly lower.

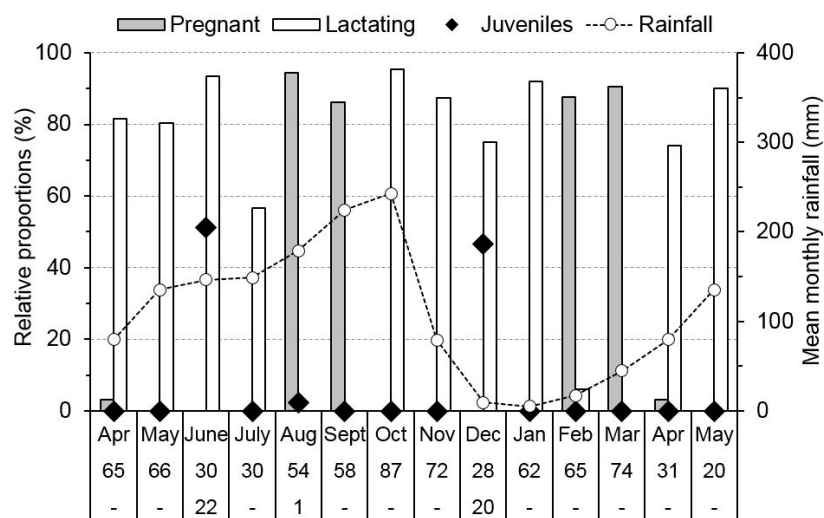


**Figure 2.** Monthly rainfall (bars) and average minimum and maximum temperatures (dashed-symbols) in Battambang (north-western Cambodia) in 2015 (grey) and 2016 (white) in relation to long term averages (black) for the province (1982–2013). All data from the Battambang Meteorological Station (13.08° N, 103.21° E).

### 3.2. Reproductive Phenology

A total of 1497 individuals of *C. plicatus* ( $\sigma$ : $\text{♀}$ ratio = 1:1.82) were captured during the study period and a monthly mean of 107 bats were examined (min–max = 99–112, SD  $\pm$  4.3) (Table S2).

With the exception of three heavily pregnant females captured in April 2015 and 2016, the majority of pregnancies were observed in February–March and August–September (99%, 225 of 228 bats) (Figure 3). These two periods were followed by high proportions of lactation from April–July (mean = 79%, min–max = 57–93, SD  $\pm$  13) and October–January (mean = 87%, min–max = 75–95, SD  $\pm$  9). Monthly percentages of lactating females were not related to rainfall ( $r_s = -0.007$ ,  $p = 0.982$ ).

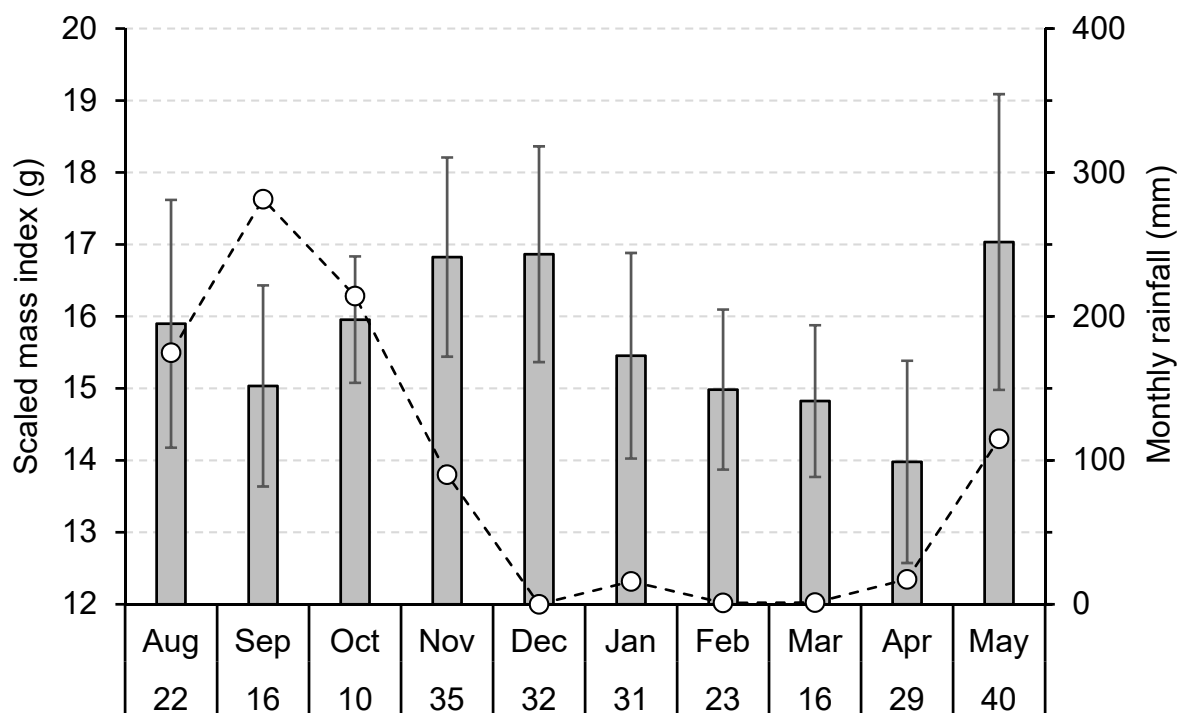


**Figure 3.** Reproductive phenology of *Chaerephon plicatus* in Battambang province (north-western Cambodia) in relation to mean monthly rainfall (1982–2013). The first row of figures below the graph represents the total number of parous, pregnant and lactating females caught each month and the second row represents the same for juveniles. Relative proportions for juveniles were derived by dividing monthly totals by the study total  $\times$  100. The figure is based on 785 bats captured from April 2015 to May 2016 inclusive.

Reproductively immature bats were encountered in every month of the study except October 2015 (monthly mean = 21, min–max = 0–46, SD  $\pm$  13). Because juvenile bats were almost exclusively observed in June and December (42 of 43 bats), two months after the onset of lactation, the majority of *C. plicatus* births evidently occur in April and October. As such, the reproductive pattern of the species in north-western Cambodia may be classifiable as seasonal or continuous bimodal polyoestry (two litters per female/year in two distinct seasons of parturition), although it is also possible that some females breed at different times of the year.

### 3.3. Seasonal Body Condition

Monthly SMI values for reproductively mature male bats ( $n = 254$ ) varied significantly from August 2015 to May 2016 ( $F = 13.293$ ,  $df = 9$ ,  $p < 0.001$ ) (Figure 4, Table S3). Mean monthly index values were not related to monthly rainfall for the same period ( $r_s = -0.03$ ,  $p = 0.934$ ). However, raw SMI values were significantly greater during the second half of the wet season (August–October 2015, mean = 15.6 g, SD  $\pm$  1.5,  $n = 48$ ) compared to the late dry season (February–April 2016, mean = 14.5 g, SD  $\pm$  1.3,  $n = 68$ ) (independent  $t = 4.086$ ,  $df = 94$ ,  $p < 0.001$ ).

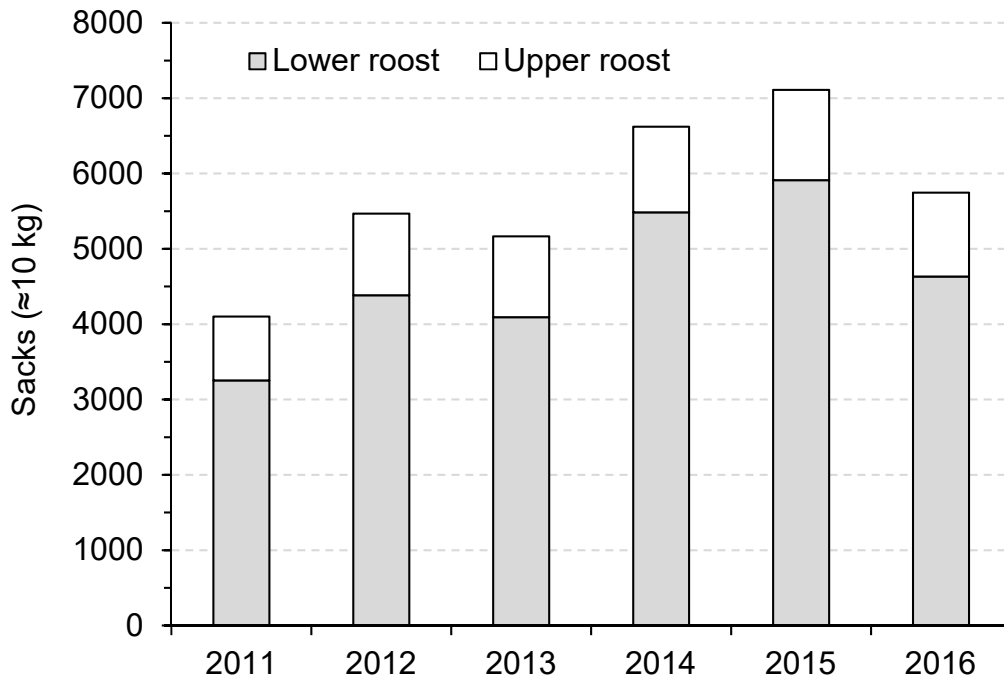


**Figure 4.** Scaled mass index (SMI) of mature male *Chaerephon plicatus* ( $n = 254$ ) in Battambang province (north-western Cambodia) from August 2015 to May 2016 (bars) in relation to monthly rainfall for the same period (broken line). Bars represent means, whiskers represent standard deviations and values below the graph are monthly sample sizes. Note the y-axis is truncated to improve resolution.

### 3.4. Guano Harvests

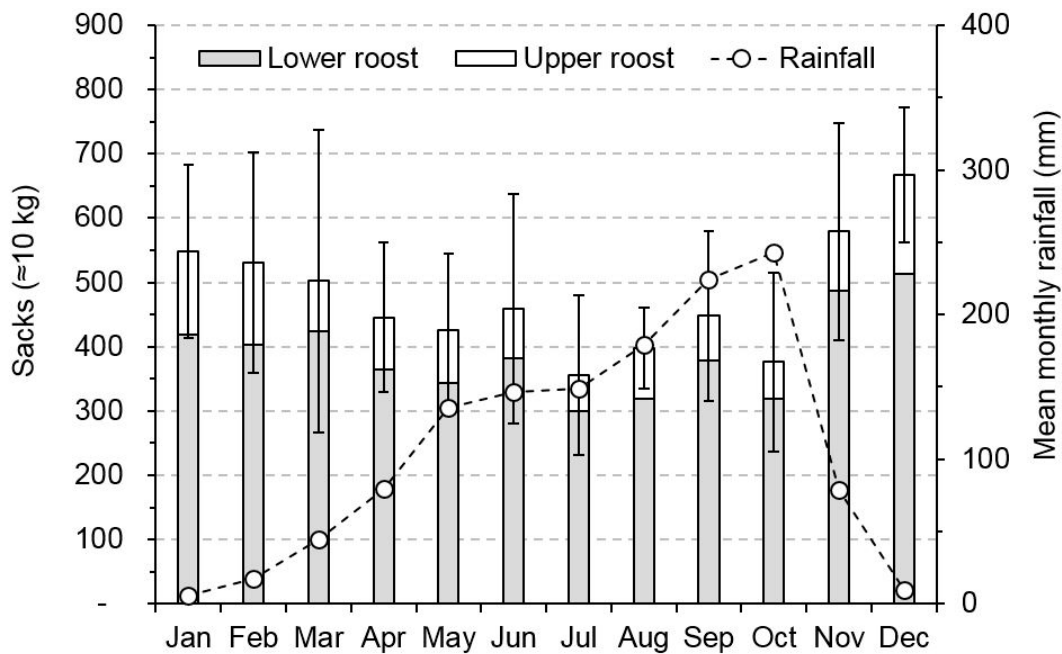
Annual guano harvests at Ta Rumm #1 cave increased almost yearly from 4101 sacks in 2011 to 7110 sacks in 2015 (73%), 79–83% of which came from the lower of the two roosts within the cave (Figure 5, Table S4). This was followed by a 19% decrease to 5746 sacks (81% from the lower roost) in 2016. These figures equate to a study nadir of 41.0 tons of guano in 2011, a study peak of 71.1 tons in 2015, followed by 57.5 tons in 2016.





**Figure 5.** Annual guano harvest from Ta Rumm #1 cave (Battambang province, north-western Cambodia), 2011–2016.

Between January 2011 and February 2017, total monthly guano harvests varied significantly across the calendar year ( $F = 2.335$ ,  $df = 11$ ,  $p = 0.018$ ) (Figure 6). Mean monthly harvests were negatively correlated with mean monthly rainfall ( $r = -0.779$ ,  $p = 0.003$ ) and so were significantly greater during the dry season (November–April) (independent  $t = 3.747$ ,  $df = 70$ ,  $p < 0.001$ ).



**Figure 6.** Mean monthly guano harvests from Ta Rumm #1 cave (Battambang province, north-western Cambodia) for January 2011–February 2017 in relation to mean monthly rainfall (1982–2013). Bars represent means and whiskers represent standard deviations.

#### 4. Discussion

Our study provides the first information on the reproductive ecology of *C. plicatus* in Cambodia. Although still abundant in at least two parts of the country, with several colonies locally protected, the tendency of the species to aggregate in large numbers in caves or buildings renders it particularly vulnerable to hunting for bush meat consumption and human disturbance [31,32]. This is demonstrated by its apparent extinction in Singapore [33] and the loss of nearly all large colonies in the Philippines, several in Laos, and at least one in Myanmar and Cambodia [25,32].

Because we observed pregnancies almost exclusively in February–March and August–September and juvenile bats in June and December, the majority of *C. plicatus* births at our study site evidently occur in April and October. As this matches the timing of births reported at other *C. plicatus* colonies in north-western and southern Cambodia (sites #3, #4 and #12, Table 1, Figure 1), our findings are likely to be applicable nationwide. This is supported by the fact that they also match the reproductive phenology of the species in south-central Thailand [16,18], and central and coastal Myanmar [17]. However, while growing evidence suggests that seasonally monoestrous insectivorous cave bats exhibit reproductive synchrony across Southeast Asia, even in areas with limited seasonality in rainfall [34], it remains unclear whether the same might be true for a species with two annual breeding cycles such as *C. plicatus*. This is due to an apparent lack of information on the reproduction of the species in range countries such as China, Vietnam, Malaysia, Indonesia and the Philippines. Nonetheless, our confirmation of critical reproductive periods for *C. plicatus* in Cambodia provides clear guidance as to when disturbance of in-country colonies, such as inappropriate guano harvesting practices, should be avoided. At a minimum, the period from late pregnancy through to weaning each year (e.g., March–June and September–December) should be regarded as critical times requiring the application of sustainable guano harvesting techniques [35].

Our finding that body condition (as indicated by the SMI) is significantly improved during the late wet season (August–October) compared to the late dry season (February–April) was expected. This is because insect biomass and thus food availability for insectivorous bats such as *C. plicatus* correlates positively with rainfall [19,20], and consequently peaks during the wet season in the seasonal tropics of Southeast Asia [36,37]. Conversely, the progressive decline we observed in the live-weights of male bats from December 2015 through April 2016 suggests that these may experience increasing energetic stress as the dry season (November–April) progresses each year. Fat depletion due to energy demanding processes, such as recrudescence of the male reproductive tract, and competitive mating during the same period could also contribute to this; although additional research is needed to confirm this. Further studies are also desirable to confirm if our findings represent a recurrent seasonal pattern. If this is the case and typical for both sexes each year however, this stress might be exacerbated for reproductive females since these are largely pregnant in February–March and bear young in April. As a consequence, precautionary principles warrant consideration of the late dry season as a period of heightened vulnerability for *C. plicatus* in Cambodia.

Although rates of guano accumulation should reflect the size of bat colonies [4], their utility as a proxy for population size at our study site was compromised by the practical difficulty of ensuring consistent harvest methods (due to safety concerns related to ammonia levels in the cave) and the fact that unquantified amounts of guano are flushed from accessible areas of the cave by percolating waters during the wet season [22]. The latter may explain why guano harvests were significantly greater in the 2011–2017 dry seasons when the opposite might be expected due to greater food availability during the wet season. Use of harvest data as a proxy for monitoring bat population size at Ta Rumm #1 cave would also be confounded by reported movements between *C. plicatus* colonies in the region, four of which occur within 10 km of the site. Notwithstanding this, the decline recorded in guano harvests in 2016 despite lower rainfall may be partly attributable to mass mortalities of cave bats observed in April that year in Cambodia [38]. This was apparently due to several areas of the country experiencing the highest temperatures on record, with an all-time national high of 42.6 °C in northern Cambodia [39]. Though some degree of mortality commonly occurs during parturition periods for *C. plicatus* [17],

the managers of three cave colonies in Battambang (#1, #3 and #4, Figure 1) reported that the levels witnessed in April 2016 were among the highest they had seen and estimated colony losses of 10–30%.

The seven cave colonies representing the bulk of *C. plicatus* populations in Cambodia have been effectively protected by local communities for several decades. Protection of the colony at Ta Rumm #1 is particularly effective as the concessionaire lives directly below the cave. We recommend these communities be encouraged to continue protecting their bat colonies through the establishment of sustainable guano harvesting practices, harvest record systems and population monitoring programs [35]. Because movement of bats between colonies has been reported by site managers/concessionaires in Battambang, studies to determine the scale, periodicity and drivers of such exchanges are also desirable. Furthermore, as the status of the five colonies identified at scattered anthropogenic roost sites in central Cambodia [11] is currently unknown, efforts to clarify this are similarly warranted.

**Supplementary Materials:** The following are available online at <http://www.mdpi.com/1424-2818/10/3/91/s1>, Table S1: Monthly rainfall and average minimum and maximum temperature data for Battambang province (north-western Cambodia), 1982–2013 and 2015–2016; Table S2: Sex, age and reproductive status of *C. plicatus* in Battambang province (north-western Cambodia) from April 2015 to May 2016 inclusive; Table S3: Live-weights of mature male *C. plicatus* ( $n = 254$ ) in Battambang province (north-western Cambodia) from August 2015 to May 2016 inclusive; Table S4: Guano harvest records for Ta Rumm #1 cave in Battambang province (north-western Cambodia) from January 2011 to February 2017 inclusive.

**Author Contributions:** N.M.F., P.A.R. and J.C. conceived the research; N.M.F., S.I. and J.C. performed the research; N.M.F., P.A.R. and J.C. analyzed the data; V.T. contributed materials; N.M.F. wrote the paper with contributions from P.A.R., I.S., V.T. and J.C.

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## References

1. Kingston, T. Response of bat diversity to forest disturbance in Southeast Asia—Insights from long term research in Malaysia. In *Bat Evolution, Ecology, and Conservation*; Adams, R.A., Pedersen, S.C., Eds.; Springer: New York, NY, USA, 2013; pp. 169–185, ISBN 978-1-4614-7396-1.
2. Sodhi, N.S.; Koh, L.P.; Clements, R.; Wanger, T.C.; Hill, J.K.; Hamer, K.C.; Clough, Y.; Tscharntke, T.; Posa, M.R.C.; Lee, T.M. Conserving Southeast Asian forest biodiversity in human-modified landscapes. *Biol. Conserv.* **2010**, *143*, 2375–2384. [[CrossRef](#)]
3. Jones, G.W. *The Population of Southeast Asia*; Working Paper Series No. 196; Asia Research Institute: Singapore, 2013; pp. 1–39.
4. Furey, N.M.; Racey, P.A. Conservation ecology of cave bats. In *Bats in the Anthropocene: Conservation of Bats in a Changing World*; Voigt, C.C., Kingston, T., Eds.; Springer: Basel, Switzerland, 2016; pp. 463–500, ISBN 978-3-319-25218-6.
5. Simmons, N.B. Order Chiroptera. In *Mammal Species of the World: A Taxonomic and Geographic Reference*, 3rd ed.; Wilson, D.E., Reeder, D.M., Eds.; Johns Hopkins University Press: Baltimore, MD, USA, 2005; pp. 312–529, ISBN 0-8018-8221-4.
6. Bates, P.J.J.; Harrison, D.L. *Bats of the Indian Subcontinent*; Harrison Zoological Museum: Kent, UK, 1997; pp. 1–258, ISBN 0-9517313-1-9.

7. Wanger, T.C.; Darras, K.; Bumrungsri, S.; Tschardtke, T.; Klein, A.M. Bat pest control contributes to food security in Thailand. *Biol. Conserv.* **2014**, *171*, 220–223. [CrossRef]
8. Cleveland, C.J.; Betke, M.; Federico, P.; Frank, J.D.; Hallam, T.G.; Horn, J.; López, J.D., Jr.; McCracken, G.F.; Medellín, R.A.; Moreno-Valdez, A.; et al. Economic value of the pest control service provided by Brazilian free-tailed bats in south-central Texas. *Front. Ecol. Environ.* **2006**, *4*, 238–243. [CrossRef]
9. Estimating the Economic Value of Insect-Eating Bats: A Case Study from Cambodia. Available online: [http://www.batcon.org/resources/media-education/bats-magazine/bat\\_article/846](http://www.batcon.org/resources/media-education/bats-magazine/bat_article/846) (accessed on 10 April 2018).
10. Bats Missing from the Museum. The Cambodia Daily, 20 March 2002. Available online: <https://www.cambodiadaily.com/news/bats-missing-from-museum-30558/> (accessed on 10 April 2018).
11. Yim, S.; Mackie, I. Status and conservation of wrinkle-lipped bat *Tadarida plicata* in Cambodia. In Proceedings of the International Meeting on “Increasing In-Country Capacity and Regional Co-Operation to Promote Bat Conservation in Cambodia, with Particular Reference to *Otomops wroughtoni*”, Phnom Penh, Cambodia, 26–27 October 2009.
12. Furey, N.M.; Whitten, T.; Cappelle, J.; Racey, P.A. The conservation status of Cambodian cave bats. In *International Speleological Project to Cambodia 2016 (Provinces of Stoeng Treng, Kampong Speu, Banteay Meanchey and Battambang)*; Laumanns, M., Ed.; Berliner Höhlenkundliche Berichte: Berlin, Germany, 2016; Volume 64, pp. 82–95, 1617–8572.
13. Racey, P.A.; Entwistle, A.C. Conservation ecology of bats. In *Bat Ecology*; Kunz, T.H., Fenton, M.B., Eds.; University of Chicago Press: Chicago, IL, USA, 2003; pp. 680–743, ISBN 0-22604620604.
14. Racey, P.A.; Entwistle, A.C. Life history and reproductive strategies in bats. In *Reproductive Biology of Bats*; Crighton, E.G., Krutzsch, P.H., Eds.; Academic Press: New York, NY, USA, 2000; pp. 363–414, ISBN 0-12-195670-9.
15. Goh, K.C. The climate of Southeast Asia. In *The Physical Geography of Southeast Asia*; Gupta, A., Ed.; Oxford University Press: Oxford, UK, 2005; pp. 80–93, ISBN 0-19-924802-8.
16. Hillman, A. The study on wrinkled-lipped free-tailed bats (*Tadarida plicata*) at Khao Chong Pran Non-hunting Area, Ratchaburi Province. *R. For. Dep. J.* **1999**, *1*, 72–83.
17. Aye, N.N. Ecology and Economic Importance of *Tadarida plicata* (Buchanan, 1800) Free-Tailed Bat in Some Areas of Myanmar. Ph.D. Thesis, University of Yangon, Yangon, Myanmar, 2006.
18. Leelapaibul, W.; Bumrungsri, S.; Pattanawiboon, A. Diet of wrinkle-lipped free-tailed bat (*Tadarida plicata* Buchanan, 1800) in central Thailand: Insectivorous bats potentially act as biological pest control agents. *Acta Chiropterol.* **2005**, *7*, 111–119. [CrossRef]
19. McWilliam, A.N. Adaptive Responses to Seasonality in Four Species of Microchiroptera in Coastal Kenya. Ph.D. Thesis, University of Aberdeen, Aberdeen, UK, 1982.
20. Nurul-Ain, E.; Rosli, H.; Kingston, T. Resource availability and roosting ecology shape reproductive phenology of rainforest insectivorous bats. *Biotropica* **2017**, *49*, 382–394. [CrossRef]
21. Laumanns, M. *International Speleological Project to Cambodia 2008 (Battambang Area)*; Berliner Höhlenkundliche Berichte: Berlin, Germany, 2009; Volume 34, pp. 1–69, 1617–8572.
22. Chheng, C.; Ta Rumm #1 Cave Concession Holder, Phnom Sampeu, Battambang Province, Cambodia. Personal communication, 2016.
23. Lunn, B.; Phnom Romseisak Community Forest Chief, Bobh Village, Bannong District, Battambang Province, Cambodia. Personal communication, 2016.
24. Sikes, R.S.; Gannon, W.L.; Animal Care and Use Committee of the American Society of Mammalogists. Guidelines of the American Society of Mammalogists for the use of wild animals in research. *J. Mammal.* **2011**, *92*, 235–253. [CrossRef]
25. Francis, C.M. *A Guide to the Mammals of Southeast Asia*; Princeton University Press: Princeton, NJ, USA, 2008; pp. 1–392, ISBN 978-0-691-13551-9.
26. Kruskop, S. *Bats of Vietnam, Checklist and an Identification Manual*, 2nd ed.; KMK Scientific Press: Moscow, Russia, 2013; pp. 1–299, ISBN 978-5-87317-901-5.
27. Anthony, E.L.P. Age determination in bats. In *Ecological and Behavioral Methods for the Study of Bats*; Kunz, T.H., Ed.; Smithsonian Press: Washington, DC, USA, 1988; pp. 47–58, ISBN 0874745969.
28. Racey, P.A. Reproductive assessment. In *Behavioural and Ecological Methods for the Study of Bats*, 2nd ed.; Kunz, T.H., Parsons, S., Eds.; Johns Hopkins University Press: Baltimore, MD, USA, 2009; pp. 249–264, ISBN 978-0-8018-9147-2.

29. Furey, N.M.; Mackie, I.J.; Racey, P.A. Reproductive phenology of bat assemblages in Vietnamese karst and its conservation implications. *Acta Chiropterol.* **2011**, *13*, 341–354. [[CrossRef](#)]
30. Peig, J.; Green, A.J. New perspectives for estimating body condition from mass/length data: The scaled mass index as an alternative method. *Oikos* **2009**, *118*, 1883–1891. [[CrossRef](#)]
31. Thomas, N.M.; Duckworth, J.W.; Doaungboubpha, B.; Williams, M.; Francis, C.M. A checklist of bats (Mammalia: Chiroptera) from Lao PDR. *Acta Chiropterol.* **2013**, *15*, 193–260. [[CrossRef](#)]
32. Csorba, G.; Bumrungsri, S.; Francis, C.; Bates, P.; Ong, P.; Gumal, M.; Kingston, T.; Heaney, L.; Balete, D.; Molur, S.; et al. *Chaerephon plicatus*. The IUCN Red List of Threatened Species. 2014. Available online: <http://www.iucnredlist.org/details/4316/0> (accessed on 20 April 2018). [[CrossRef](#)]
33. Lane, D.J.; Kingston, T.; Lee, B.P.H. Dramatic decline in bat species richness in Singapore, with implications for Southeast Asia. *Biol. Conserv.* **2006**, *131*, 584–593. [[CrossRef](#)]
34. Lim, T.; Cappelle, J.; Hoem, T.; Furey, N.M. Insectivorous bat reproduction and human cave visitation in Cambodia: A perfect conservation storm? *PLoS ONE* **2018**, *13*, e0196554. [[CrossRef](#)] [[PubMed](#)]
35. IUCN-SSC. *IUCN-SSC Guidelines for Minimizing the Negative Impact to Bats and Other Cave Organisms from Guano Harvesting, Ver. 1.0*; IUCN Species Survival Commission: Gland, Switzerland, 2014; pp. 1–12.
36. Ades, G.W.J.; Dudgeon, D. Insect seasonality in Hong Kong: A monsoonal environment in the northern tropics. *Mem. Hong Kong Natl. Hist. Soc.* **1999**, *22*, 81–97.
37. Kai, K.H.; Corlett, R.T. Seasonality of forest invertebrates in Hong Kong, South China. *J. Trop. Ecol.* **2002**, *18*, 637–644. [[CrossRef](#)]
38. Heat Wave Blamed as Hundreds of Bats Die in Siem Reap. The Phnom Penh Post, 22 April 2016. Available online: <https://www.phnompenhpost.com/national/heat-wave-blamed-hundreds-bats-die-siem-reap> (accessed on 10 April 2018).
39. Scorching Hot Season Sets New All-Time Highs. The Phnom Penh Post, 21 April 2016. Available online: <https://www.phnompenhpost.com/national/scorching-hot-season-sets-new-all-time-highs> (accessed on 10 April 2018).



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