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Brumation of the clouded monitor lizard *Varanus nebulosus* in north-eastern Thailand

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ABSTRACT - The clouded monitor lizard (*Varanus nebulosus*), is a semi arboreal lizard widely distributed throughout much of South and Southeast Asia. Despite its wide distribution there is almost nothing known about the ecology of this species. During the course of an 11-month radio telemetry study, in a reserve with a tropical savannah climate (Köppen Aw), we made the first records of brumation in this monitor lizard. This contrasts with earlier reports of the same species in a tropical monsoon climate (Köppen Am) where no brumation was recorded. We successfully tracked 10 individuals throughout their inactive period and found that seven of the monitors selected tree hollows within the endangered *Shorea henryana* tree. All tree hollows selected faced between the east and south cardinal points (90 °-180 °). The average brumation period was 100 days (range 86-113 days, standard deviation- 10.7), beginning in November at a time of falling temperatures and humidities and ending in early March when these variables had been restored. Eight of the 10 monitors basked partially or completely out of their shelters on multiple occasions. Of those eight monitors, two individuals moved between shelters during brumation after an extended period in one location. Our observations provide insight into the relationship between *V. nebulosus* and the tree *S.henryana*, in the dry evergreen forests of north-eastern Thailand. Future research should investigate how this tree will be affected by climate change in the coming decades and what that could mean for the future persistence of the clouded monitors that appear to rely on it.

INTRODUCTION

Brumation is a form of hibernation in ectothermic animals, defined as a period of inactivity or dormancy, typically associated with changes in environmental temperatures (Mayhew, 1965). Environmental temperature plays a critical role in the ability of ectothermic species to: capture prey, avoid predation and regulate their metabolism (Mcconnachie & Alexander, 2004; Naulleau, 1983; Stevenson et al., 1985). Brumation and hibernation are well documented in reptiles, with many temperate species spending two-thirds of their lives inactive (Etheridge et al., 1983). Despite these cycles being such a large part of an animal's life-history, there is little understanding of the drivers leading to their onset and subsequent emergence, especially for species in the tropics.

The clouded monitor lizard *Varanus nebulosus* (Gray, 1831) is a semi-arboreal well camouflaged (low detection rate) species with a wide distribution from Myanmar to Vietnam and southern China to Indonesia (Koch et al., 2013). They are a medium-sized varanid reaching a maximum length of 160 cm and maximum weight of 8 kg (Auffenberg, 1994). Their conservation status in the wild has yet to be assessed and the IUCN listing remains under Bengal monitor (*Varanus bengalensis*) as there is uncertainty among researchers pertaining to the species status. Numerous studies suggest two separate but closely related species (sister species) apparent from; distinguishable oblique ventral scale counts (*bengalensis* 88-110, *nebulosus* 70-90; Auffenberg, 1994), distinct hemipenal differences (Ziegler & Böhme, 1997), differences in scale morphology and micro-structures (Bucklitsch et al., 2016), and mitochondrial DNA (Ast, 2001). It is suggested that since the number of mature individuals is steadily declining, they will require a "vulnerable" listing once properly evaluated (Cota et al., 2021). Despite their large size and broad geographical distribution, the general ecology of the clouded monitor remains little known (but see Traeholt, 1997; Duengkae & Chuaynkern, 2009 for records on diet and basking behaviour).

Radio telemetry is a commonly accepted method to record seasonal and daily activity patterns, home range sizes, movement trajectories, macrohabitat use, shelter site preferences and ultimately threats to survival (Cagnacci et al., 2010; Malhotra et al., 2021; Ujvari & Korsos, 2000). The objective of this study was to observe whether the clouded monitor in north-eastern Thailand has an annual period of brumation and if so then address the following questions: a) When does brumation occur and what is it's duration? b) Do the monitors select specific species of trees for brumation? c) What specific microhabitat features do they select? d) Do the monitors have a dormant or active brumation (dormant equating to no movement at all; active consisting of thermoregulating and possibly moving between sites)? and, e) Does average daily temperature and humidity contribute to either the onset of, or emergence from, brumation?

METHODS

Study Site

From July of 2020, we undertook an 11-month radio telemetry study of the clouded monitor at the Sakaerat Environmental

Research Station in north-eastern Thailand. The research station is a part of the Sakaerat Biosphere Reserve (SBR; 14.44-14.55 °N, 101.88-101.95 °E), encompassing an area of approximately 360 km^2. The biosphere reserve has three main designations: core, buffer and transitional. The core area is predominantly dry evergreen forest (DEF) with large patches of dry dipterocarp forest (DDF) and has active ranger enforcement; the buffer zone consists mainly of both DEF and plantation forest; the transitional zone lacks official protection and is an agricultural matrix with expanding human settlements (Trisurat, 2010). The DEF at SBR, has a mean canopy height of 35-40 m with two subtypes dependent upon the dominant tree species: *Hopea ferrea* dominates the first type and occurs on level ground creating a closed canopy; *Shorea henryanna*, dominates the second type and mainly occurs on slopes, creating a patchy canopy (Bunyavejchewin, 1986, 1999). Both *H. ferrea* and *S. henryana* are classified as endangered species and are at risk from habitat loss and logging (Ly et al., 2017a & b). In contrast, the DDF at SBR, has a mean canopy height of 11-14 m and a more open canopy. Several species, *Shorea roxburghii*, *Shorea obtusa* and *Dipterocarpus intricatus* are dominant trees in DDF (Lamotte et al., 1998). Based on the Köppen climate classification, SBR is a tropical savanna (Aw) with an altitude range between 280-762 m a.s.l. (Köppen, 1931; Rubel & Kottek, 2010). The SBR has three distinct seasons: dry (November-February), hot (March-May) and wet (May-October; Tantipanatip et al., 2016).

Capture/Tracking Techniques

Monitors were located during road-cruising on forest roads with a motorcycle and visual surveys by scanning large trees with binoculars (Fig. 1). Once found, we captured monitors either with drop traps - placed along the base of the tree with a sheet metal perimeter - or with a noose. Once captured the monitors had a backpack style harness fitted around their pelvic girdle, which contained a radio transmitter and GPS logger. We released all individuals at the exact capture site and began radio tracking them once daily between 05:00 h and 20:00 h. During this study we recorded a considerable brumation period for all study animals - 8 individuals - beginning on 5th November 2020 and lasting until 6th March 2021 (date of last emergence). We defined the onset of brumation as the first date from which a lizard remained in a shelter site for at least 14 days, during a period where we deemed the weather adequate for movement. Although we did record large spans (up to 13 days) of inactivity within our study animals, prior to brumation, these were all during the monsoon season, through periods of heavy rainfall, high cloud cover and lower temperatures.

We also found three new individuals during the brumation period: M11 on 18th November, M12 on 27th November and M13 on 27th December, 2020. The first two monitors were basking outside of their brumation sites, so we placed traps along the shelter-tree base. The third individual was found on a forest road, perhaps moving from one shelter site to the next. We documented two of our radio tracked monitors moving between brumation sites around the same timeframe: F01 on 19th December and F09 on 21st December, 2020. The traps set for M11 and M12, were checked daily during radio tracking protocols and observations of basking were documented, when possible, on these two individuals. Although we do not know what date these two monitors entered brumation we decided to include them in our dataset because we could determine the exit dates, and found each within the range of our other radio tracked monitors entering brumation. However, because M13 was captured late into the brumation cycle we have chosen not to include it in our summary dataset.

Figure 1. Clouded monitor F03 basking completely exposed above its hollow in a *Hopea ferrea* tree

Data Collection

During radio tracking we recorded: brumation site location, the onset date, the frequency of basking observations (recorded during each daily fix if the lizard was visible when we were using telemetry to find the individual) and the date of emergence from brumation of each monitor (Fig. 2). We assessed each monitor's shelter site location by recording: habitat type, species of the tree selected, the diameter at breast height (DBH) using a tape measure, the height of the shelter site and height of the tallest branch (both measured using a Nikon Forestry Pro I rangefinder) as well as the cardinal direction the shelter site was facing measured using the compass feature on a handheld Garmin 64s GPS.

There are five weather stations spread throughout the SBR; each station records the atmospheric temperature and humidity every hour. We collated station weather data and averaged all of the stations together to plot the average temperature and humidity for our study period. We then overlaid the monitor's dormancy duration, to see if there were any relationships between these two climatic factors and the onset of, or emergence from, brumation (Fig. 3).

We used R studio version 4.0.3 (R Core Development Team, 2020) for data manipulation with reshape2 package version 1.4.4 (Wickham, 2007) data visualizations including ggplot2 version 3.3.5 (Wickham, 2016). To produce temperature and humidity graphics we employed ggpubr version 0.4.0 (Kassambara, 2020) for final visuals, lubridate version 1.8.0 (Grolemund and Wickham 2011) to control date formats, tidyverse version 1.3.0 (Wickham et al. 2019) for functionality, and viridis version 0.6.2 for colour-blind friendly palettes. We have additionally made all data and R scripts available on Open Science Framework (https://osf.io/xd243/).

RESULTS

Brumation sites

For the 10 monitors we followed through brumation, we recorded 14 different brumation sites. All study animals spent the duration of brumation within tree hollows, despite differing habitat types. Of the 14 shelter sites documented, 64% were on *S. henryana* trees. We were unable to identify two tree species (used by M04 and M07), because both trees were already dead with no identifiable features left. The average shelter site height was 21.6 m above the ground (range - 2.7-39.6, standard deviation - 10.8; Table 1). The orientations of the tree hollows were all within the east and south cardinal points (90 °-180 °) and occurred within gaps or clearings of the canopy, allowing open access to direct sunlight (Table 1). All shelter trees were large (DBH mean - 252.2 cm, range - 102.5-326.7, standard deviation - 59.6; Table 1). We were unable to take measurements on the depth, width and internal features of the tree hollows due to the heights of shelter sites.

Table 1. Characteristics of the brumation sites used by *Varanus nebulosus*. Shelter height is the height of the tree hollow utilized by each monitor. Tree height is the height of the tallest branch. DBH is the diameter breast height (girth) of the bole of each tree. Shelter direction is the cardinal direction of the tree hollow selected by each monitor.

Monitor ID	Forest habitat	Tree species	Shelter height (m)	Tree height (m)	DBH (cm)	Shelter direc-tion
F01	MDF	*Shorea henryana*	19.1	27.3	254.4	E
F01*	MDF	*Lagerstroemia calyculata*	21.4	29.8	287.3	E
F03	DEF	*Hopea ferrea*	14.4	17.5	198.6	ESE
M04	HS	Unknown	2.7	2.7	102.5	E
M06	DEF	*Shorea henryana*	26.3	35.7	274.2	ESE
M07	DDF	Unknown	11.4	11.4	190.6	E
M08	DDF	*Pterocarpus macrocarpus*	11.8	21.5	263.2	E
F09	DEF	*Shorea henryana*	19.0	26.7	298.8	SE
F09*	DEF	*Shorea henryana*	37.9	41.2	301.3	ESE
F09*	DEF	*Shorea henryana*	18.3	28.8	243.9	E
F09*	DEF	*Shorea henryana*	21.2	33.2	260.1	ESE
M10	DEF	*Shorea henryana*	38.3	43.9	326.7	SE
M11	DEF	*Shorea henryana*	39.6	45.6	312.5	SE
M12	PLT	*Shorea henryana*	20.6	31.1	216.0	SSE

Habitat Abbreviations: DEF=Dry Evergreen Forest, DDF= Dry Dipterocarp Forest, MDF= Mixed Deciduous Forest, PLT= Plantation Forest and HS= Human Settlement

Shelter Direction Abbreviations: E = East, ESE = East by Southeast, SE = Southeast, SSE = South by Southeast Asterisks (*) indicate when individual monitors moved from one shelter site to another during the brumation period.

Brumation observations

There was a distinct relationship between the onset of brumation with lower average temperatures and humidity. We observed that the first study animals entered brumation shortly after the average daily temperature dropped below 22 °C (Fig. 3). Brumation peaked during the lowest average temperature and humidity for the year. Individuals brumated on average for 100 days (range - 86-113 days, standard deviation - 10.7; Fig. 2). All clouded monitors entered brumation within 29 days of each other and emerged within a 12-day span (Fig. 2). We observed that all but two individuals (M07 and M10) basked either partially or completely out of their tree hollows (Figs. 1&2).

Both monitors F01 and F09 moved to different shelter locations during the course of brumation. Monitor F01 moved once on 19th December 2020, from a *S. henryana* tree to a *Lagerstroemia calyculata*. Monitor F09, moved three times through the brumation cycle, moving to a *S. henryana* each time, on: 21st December 2020, 29th January 2021 and 12th February 2021 (Table 1; Fig. 2).

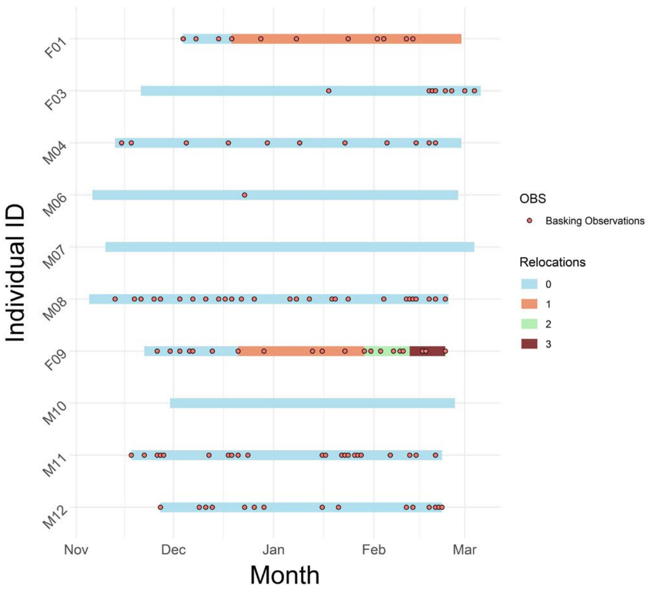

Figure 2. Brumation observations of clouded monitor (*Varanus nebulosus*). Most monitors entered brumation in November 2020 and all individuals had completely emerged by early March 2021. Relocations are annotated for monitors which moved to different shelter sites during brumation. Basking observations are listed for each visual observation we made during this period.

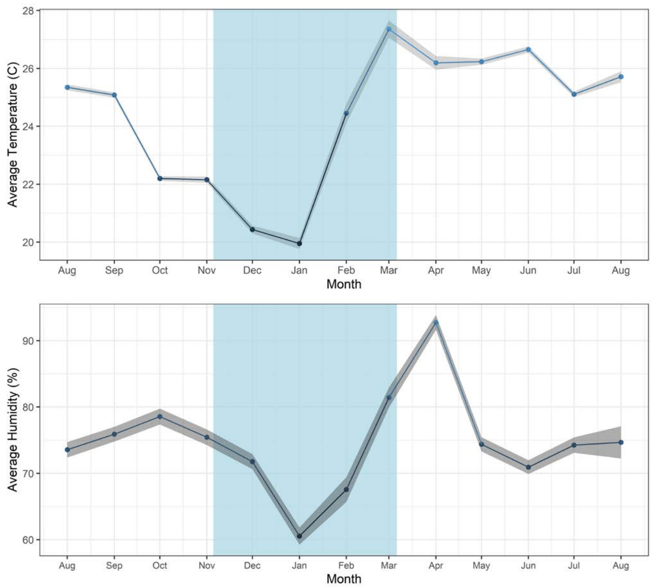

Figure 3. Climatic conditions at the Sakaerat Environmental Research Station from August 2020 to August 2021. Top-Mean daily temperature with upper and lower confidence intervals, Bottom - mean daily relative humidity (with upper and lower confidence intervals). In both cases the shaded blue area is the total brumation period, 6th November 2020 to 6th March 2021.

DISCUSSION

Brumation behaviour

Walter Auffenberg, in his seminal work on the Bengal monitor (*V. bengalensis*), recorded individuals from Trang province in Thailand (now considered *Varanus nebulosus*), that did not enter a period of inactivity and instead remained active throughout the year (Auffenberg, 1994).The climate of Trang province by the Köppen classification is tropical monsoon (Am), with distinct wet and dry seasons and an almost uniform temperature throughout the year (Rubel & Kottek, 2010). We have potentially demonstrated variation between *V. nebulosus* populations within the same geographical region. It is likely that *V. nebulosus* goes through a brumation period throughout the northern part of its distribution where there is a tropical savannah climate (Köppen Aw) with a distinct cool dry period and that it remains active throughout the year in the southern part where there is a tropical monsoon climate (Köppen Am).

Shelter selection

We saw that throughout the brumation period most study animals selected tree hollows within *S. henryana* trees. Of the five monitors living within the DEF, four selected *S. henryana*. Also, two monitors living in the MDF and plantation forest also sought out this same species of tree within habitats where it is uncommon (Bunyavejchewin, 1999). Monitors within the DDF and HS each selected different species of trees and this may be due to the forest structure: in the DEF the canopy is mostly closed, so *S. henryana* provides a good shelter site for obtaining adequate UV radiation for thermoregulation; the DDF and HS have an open canopy and therefore monitors inhabiting these areas may have a broad range of potential shelter sites. Within the SBR, mature *S. henryana* individuals are rare and have high mortality rates as young trees (Bunyavejchewin, 1999). However, climate change in the tropical dry forests of north-eastern Thailand, likely will lead to a shift to wetter tropical forests by the year 2100; in this scenario the density of *S. henryana* trees are likely to decrease at a faster rate than at present (Boonpragob & Santisirisomboon, 1996). Based on our results it is likely that *S. henryana* is a critical species for *V. nebulosus*, playing a key role in their brumation cycle and possibly their overall survival in DEF. Future research should investigate how *S. henryana* will be affected by climate change in the coming decades and what that could mean for the future persistence of *V. nebulosus* at this site.

Brumation behaviour

Every tree hollow that the tracked monitors selected faced between the east and south cardinal points and all shelter sites were fully exposed, either above or within canopy gaps. This feature may be a necessity for survival during the cold season as we observed individuals on numerous occasions thermoregulating on warm mornings and afternoons. Clark et al. (2008) found that rattlesnakes selected over-wintering hibernacula on south-facing slopes, which is likely due to southern slopes receiving more solar radiation than north-facing slopes (Hamilton & Nowak, 2009). This same principle is likely the basis for *V. nebulosus* selection of east and south facing tree hollows. East and south basking orientation has also been observed in agamid lizards in Saudi Arabia (Al-Johany, 1995) and in arboreal skinks in Brazil (Maia-Carneiro et al., 2018).

Two individuals moved between shelter sites during the brumation period. F01 moved from a *S. henryana* to a *Lagerstroemia calyculata* tree. F09 changed shelter sites three times and all three occasions F09 moved to a different *S. henryana* tree and never moved more than 100 m. Cummings (2020) documented a single female desert tortoise which also moved between shelter sites during its brumation period, while all other individuals monitored remained stationary. These observations could have been linked to uncommonly warm days, or perhaps insufficient basking area on the original trees.

In most reptile species documented that brumate or hibernate, the males emerge before the females (Etheridge et al., 1986; Winck & Cechin, 2008). Although with *V. nebulosus*, the only two individuals to move between shelter sites were females and based on the emergence dates we saw no clear distinguishing patterns between male or female emergence. We were unable to collect any microhabitat data which could help deduce why these monitors moved during brumation because shelter sites were too high to safely reach without climbing gear (which we lacked). We assessed the average and daily high temperatures between 18th December and 27th December 2020 since we documented three different individuals (F01, F09 and M13) moving in this period. However, we were unable to come to any clear conclusion about what was driving this behaviour, suggesting there are other unknown underlying factors.

Potential drivers of brumation in the tropics

We observed average daily temperature and humidity at our study site during our study period and were able to identify a marker for the onset of brumation - when the average daily temperature fell below 22 °C combined with the average daily humidity dropping below 75 %. However, when the temperature and humidity rose above average in late January, the monitors all remained dormant suggesting that there are potentially other factors at play. These could include daily photoperiod, prey abundance, rainfall, peak daily temperatures, or a combination of factors (Auffenberg, 1994; Ortiz et al., 2016).

It is imperative to understand the underlying drivers of animal behaviours as the effects of climate change are predicted to alter the structure of many forests around the globe (Boonpragob & Santisirisomboon, 1996). Understanding environmental drivers and microhabitat features utilised by different species is important for planning and implementing effective conservation management (Ljubisavljević et al., 2017). We observed a clear relationship between the tree *S. henryana* and *V. nebulosus* at the SBR; it is important to determine whether this relationship persists throughout north-eastern Thailand. If so, what are the implications for populations of *V. nebulosus* in areas where *S. henryana* has been extirpated or severely decreased? It would also be worthwhile identifying the specific factors that lead ectothermic species to brumate when they are living in tropical climates with relatively little annual temperature variation (as in the current study). Despite the limited scope of our study, our preliminary data demonstrate behaviour that has not been widely documented and our findings can be used to further bolster understanding of the life-histories of ectothermic species in tropical environments.

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