

Title of project	Selection and characteristics of the reticulated pythons sleeping sites within the Lower Kinabatangan Wildlife Sanctuary.
Name	Olivia Jayne Fitzpatrick
Degree scheme	Biological sciences
Name of vocational supervisor	Prof Benoit Goossens
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Abstract

Very little research has been done examining the sleeping sites of reticulated pythons once they have passed the juvenile stage. Reticulated pythons are generalists and have appeared to thrive in the fragmented landscape of the Lower Kinabatangan Wildlife Sanctuary in Sabah (Malaysia). In this study we provided baseline data on the selection and characteristics of their sleeping sites.

Sleeping sites were located from previously VHF tagged pythons. Habitat assessment was carried out in 50m² quadrats set up around the pythons previous location. Log number, sapling count, vegetation density, ground type, canopy cover and canopy height and the distance from the python of the four closest trees were recorded, offsite the distance of the sites from the Kinabatangan river was measured using QGIS. These recordings were then repeated 50 m away in a random direction, these random sites served as an example of where the pythons could have slept but decided not to. Statistical analysis was performed with R studio using a binomial generalised linear model to compare the sleeping sites and random sites, to discover if there were any significant differences between the two categories that could highlight a selection preference.

Our findings show that reticulated pythons were actively selecting their sleeping sites for high levels of vegetation density at chest height, lower levels of canopy cover and the presence of logs. These results suggested that the reticulated pythons could have been selecting for cover and protection while they sleep, and as these parameters are readily available in the Lower Kinabatangan Wildlife Sanctuary, their continued survival is likely.

Reflection

My 8 months spent at my placement in Danau Girang Field Centre has taught me many valuable lessons and has truly been a once in a lifetime opportunity. One of the main skills I've developed is how to efficiently work in a stressful situation. Assisting in collaring procedures meant I had to pay close attention to everything that was going on, and make sure I was ready to assist the Vet with anything she needed. Day to day life at DGFC taught me how to VHF and UHF track, set up camera traps and how to analyse the footage using digicam, bait for animals and through my own project how to perform habitat assessments. While doing this I got to work with an amazing team of people and learnt about some really interesting research from the resident PhD students and visiting researchers. I would work 7 days a week with very inconsistent hours, with our work schedule being announced at 8pm the night before. This meant I was able to greatly improve my time management skills when fitting in my non-scheduled work load into the day.

There were times where staying at the field centre for prolonged periods of time was stressful, especially as it was quite unclear when we were allowed to leave and having my supervisor 7 hours away where communication was only available through unreliable WIFI could make things difficult. But there was amazing support on site especially from Dr Miriam Kunde and PhD student

Richard Burger, with the help of Richard I also gained a much greater understanding of R and how best to use it. I got to experience a different culture and working with a language barrier has greatly improved my communications skills, I also got some experience in ecotourism working with field courses and visitors and while these were not always my favourite activities to do, they definitely improved my customer service skills. I also got some very unexpected experience, the Deadly 60 film crew visited during my first month on placement allowing me to observe and learn a bit about how documentary style filming takes place.

Having to leave four months early in March due to Coronavirus meant I was unable to finish my fieldwork. I still had sites left to analyse and a big part of my project, which was going to be comparing older and newer sites was not possible. Leaving early also meant the support I received while writing up my report was all via email, this made it much harder when I was having problems with statistical analysis then it would have been if I had still been at the field centre, especially when the time difference meant I sometimes had to wait several days for a response.

Overall my placement was a very well-rounded experience which has given me a much more realistic idea of how ecological research takes place, the challenges that come with it and how long it can take to complete the research.

Introduction

Reticulated pythons (*Malayopython reticulatus*) are very successful generalists (Natusch *et al* 2015). They are mostly terrestrial snakes that can often be found near rivers (Stuebing *et al* 2014) and although there is very little known about adult sleeping sites, juveniles can often be spotted sleeping on the branches of trees overlooking the river (Stuebing *et al* 2014). In general, pythons are difficult to locate and survey in both terrestrial and urban landscapes (Natusch *et al* 2015), which is likely the reason why very little research has focused on their ecology or their preferences for sleeping sites. For many animals sleeping site selection is driven by predator avoidance (Seiler *et al* 2013), the reticulated pythons main predators include pigs, serpent eagles, crocodiles and other carnivores (Mullin *et al* 2009).

The reticulated python is a nocturnal snake that can reach lengths of up to 10 metres long making it the longest snake species in the world (Stuebing *et al* 2014). The reticulated python has an attractive and unique skin pattern which has made it very popular in the fashion industry. This popularity has created a heavy exploitation and international trade of this snake, making it the most economically important large reptile in the world (Auilya *et al* 2002). Despite this heavy exploitation evidence has shown it is very unlikely to result in the extinction of reticulated pythons, although it has been shown to have a larger effect on population levels in highly fragmented habitats (Shine *et al* 1999) such as the Lower Kinabatangan Wildlife Sanctuary.

The Reticulated python has been assessed by the International Union for Conservation (IUCN) and has been ranked as least concern. It is currently listed under CITES Appendix II, meaning it is not threatened with extinction, but its trade is controlled to avoid any future threats. However, continued fragmentation of its habitat could have greater effects on its survival rate in the future, which is why further research into this relatively unknown species should be carried out.

Although the reticulated python can be found throughout southeast Asia (Rajeshkuma *et al* 2015), a region where the highest rate of major tropical deforestation has been reported (Sodhi *et al* 2004), this study looked exclusively at reticulated pythons found within the Lower Kinabatangan Wildlife Sanctuary (LKWS) in Sabah, Malaysian Borneo. The reticulated python are a protected species in Sabah, Borneo so hunting and trade is closely monitored by the wildlife authorities (Sabah Wildlife Department 1997).

The LKWS is located in the Lower Kinabatangan Floodplain which is Sabah's largest floodplain (Boonratana 2000). Large scale land conversion from forest to monocultural oil palm plantations has resulted in a highly fragmented landscape. In Malaysia, Sabah accounts for 28.6% of its oil palm production making it Malaysia's largest oil palm state (Abram *et al* 2014). The remaining forest blocks are additionally often encroached by illegal logging and other land use (Gaveau *et al* 2014), which has brought wildlife closer to human-dominated landscapes and has created an increased threat to poaching. It is now more important than ever that we understand the adaptability of the species to these rapid landscape changes, so we are able to develop appropriate conservation management strategies.

In other snake species it has been found that habitat selection is active. A study into Burmese pythons (*Python molurus bivittatus*) habitat preferences showed that the habitat selection was not random, but this selection appeared to be driven by prey presence (Hart *et al* 2015). However, prey presence is unlikely to be as important in sleeping site selection as it is in habitat selection, if a selection is detected the python has likely selected for other currently unidentified environmental factors. A study that looked at habitat selection in black rat snakes (*Elaphe obsoleta obsoleta*) found that they actively selected habitats and appeared to thrive in a fragmented landscape similarly to reticulated pythons, however this was driven by thermoregulation (Blouin-Demers 2001), which in a tropical climate is not necessarily as important for reticulated pythons. A study on the habitat use of Latastes vipers (*Vipera latastei*) found a seasonal preference where they preferred a closer proximity to water (Brito 2003) which could also be observed in reticulated pythons as our research was carried out along the Kinabatangan river.

The aim of our study was to characterise the sleeping sites of reticulated pythons (*Malayopython reticulatus*) in the LKWS and to distinguish if their selection of sleeping sites was random or active. We planned to accomplish this by analysing the sleeping sites of previously tracked reticulated pythons in the LKWS and comparing these with random sites in the same area. We measured

various different environmental factors and compared the results from the python sleeping sites and the random sites to see if there were any significant differences between the two categories, and if so how these factors differed between random and sleeping sites. We hypothesise that Reticulated pythons are actively selecting sleeping sites and if this is the case these selection preferences could be very beneficial to future research into the reticulated pythons use of its habitat.

Method and materials

Description of the study site

The sleeping sites were identified and GPS data made available by PhD candidate Richard Burger, who is currently researching the home range of reticulated pythons. Between the years of 2016-2019 five reticulated pythons (some translocated and some local) were tagged and tracked using a VHF receiver. Tracking took place during the morning between 7am – 11am while they were asleep, their

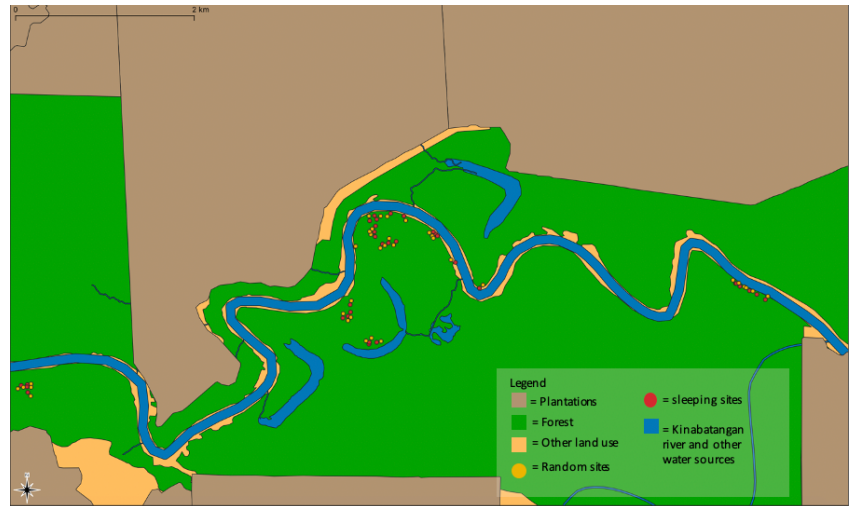


Figure.1 A map created using QGIS (version 3.6.0) of our study site within the LKWS showing the random and sleeping sites along the Kinabatangan river in the forested areas.

microhabitat was described, and their approximate location was recorded using a GPS. These sleeping sites were within the LKWS (5°10'-5°50'N, 117°40'-118°30'E) in Sabah, Borneo. All of the sites were close to the Kinabatangan river and many were close to oxbow lakes. The areas we sampled were all within the proximity of oil palm plantations but all sleeping sites were either located in the forest or on the river bank. Forests within the LKWS include semi-inundated forest, swamp forest and mangroves (Hai *et al* 2001). Some sites were within 400 metres of the field centre and others were along walking paths so there would have been human activity (other than during the hours of tracking) at these sites. All the sites analysed were lowland and susceptible to flooding, fieldwork within the sleeping sites took place between January 2020 and March 2020, during the rainy season.

Site assessment

An assessment of the area was performed at each sleeping site. At each site a 50m² quadrat was erected with the pythons GPS position in the centre, a compass was then used to set up the corners of the quadrat in the four cardinal directions. The centre and the corners of the quadrat were marked with flags. Within this quadrat the habitat parameters measured were; sapling count (with saplings being defined as between 0.5 – 3 m tall), vine presence, log count (logs required a

diameter of 10 cm and a length of 4 m), tree count (trees required a DBH of 10 cm)(DBH = diameter at breast height). Sapling count was performed first to ensure accuracy in case saplings were stepped on during other measurements. Photos of canopy cover were taken just above head height from all four corners of the quadrat and at the centre of the quadrat. Vegetation density was recorded on all four corners of the quadrat.

Vegetation density was measured using a vegetation density stick with 50 black markings, a photo was taken from the opposite side of the quadrat for all four corners (10m away) with the stick being held at both chest and ankle height. A percentage for vegetation density was measured for each corner (by counting the number of black lines visible on the density stick) and an average vegetation density of the site was taken from all four corners. All photos were taken by the same individual to ensure they were taken from the same height. On top of tree count the reticulated pythons distance from the four closest trees (greater than 10cm DBH) was recorded, these trees did not have to be within the quadrat and the DBH and canopy height was recorded for all four trees. The canopy height was calculated using a clinometer to determine the angle and the equation:

$$\text{canopy height} = \frac{((\text{TAN}(\text{RADIANS}(\text{ANGLE}^{\circ}))) \times \text{DISTANCE FROM TREE (CM)} + \text{EYE HEIGHT (CM)})}{100}$$

For each sleeping site assessed we also repeated the same method for a random site where a reticulated python was not recorded sleeping. Random sites were selected using a random number generator between 1 to 8 to determine which direction we would travel in (N, NE, E, NS, S, SW, W, NW). If this was not possible due to the river, a new number would be generated. We would then travel 50 m away from the pythons sleeping site to take a new GPS point and set up a new 50 m² quadrat around the 50 m mark. We treated the centre of the new quadrat as where the snake could have slept when measuring the distance from the Kinabatangan river and from the four closest trees.

Data analysis

Image J was used to calculate the percentage of canopy cover for each picture with an average of the five photos being recorded for each site and QGIS (version 3.6.0) was used to measure the distance from the sleeping and random sites to the Kinabatangan river. A binomial generalised linear model was created with site type (python or random) as the categorical dependent variable and the habitat parameters as the explanatory variables. The model was refined using stepwise deletion via the chi-squared method, which removed the non-significant variables using the function drop1. Mcfadden's pseudo-R² (McFadden 1979) was used to test the models goodness of fit and the results were produced using the summary function. A prediction data frame was attempted using the packages effects (Fox *et al* 2019) and ggplot2 (Wickman 2016), this was done to model if a sleeping site could be located at different percentages of canopy cover, vegetation

density at chest height and at different log counts. A generalised linear mixed model was also attempted using the significant variables from the binomial GLM, to try and distinguish if any individual preferences made by a python were masking the overall trend as there was not an equal number of sites for each of the five pythons. All statistical analysis was carried out using the program RStudio (version 1.3.1073 2020).

Covid-19 interruption

Of the 66 sites located between the years 2016 to 2019, there were 23 sites that had not yet been visited and assessed. There had also been six more reticulated pythons tagged between March – May 2020 for which sleeping sites were recorded. At these sites, further analysis of the logs present was going to take place including taking measurements of the logs, DBH, length, entrance width and its height off of the ground.

A comparison between the more recent sites and the older sites would have also taken place. We would have aimed to create a model to see whether there were significant differences between the older and newer sites. This would have helped to determine how reliable the older sites are in characterising reticulated python sleeping sites, or if change in those areas had been too great. Unfortunately, all of this was not possible due to the Covid-19 pandemic and our return to the United Kingdom mid-March 2020.

Results

Of the 44 sites visited, seven sites were deemed too changed to analyse. A total of 36 python sleeping sites and 36 random sites were analysed covering the home ranges of five reticulated pythons.

GLM

A binomial GLM (link = "logit") found that there was a significant difference between random and sleeping sites. Stepwise deletion (drop1) found that vine presence, vegetation density at ankle height, distance to the Kinabatangan river, tree number, canopy height, distance to trees and sapling count had no significant association with site selection as there was minimal variation between the two categories. The summary of the binomial GLM before stepwise deletion had a null deviance of 97.04 and a lower residual deviance of 63, showing that there was a relationship between the explanatory variables and the dependent variables; it also had an AIC of 87. The summary after stepwise deletion also had a lower residual deviance with a null of 97.041 and a residual deviance of 73.742. It also had a lower AIC of 81.742 showing the removal of insignificant variables has improved the model. Our Mcfadden's pseudo-R² was 0.246, a result over 0.2 tells us that the model fit well.

The model showed that sites with higher levels of vegetation density at chest height had a higher probability of it being a sleeping site with a P value of 0.01015. Sites with a higher log count had a higher probability of being a sleeping site with a P value of 0.03350. However, sites with higher levels of canopy cover had a higher probability of being random sites with a P value of 0.02138. All of these results show a significance of <0.05 meaning there is a low risk that these interactions occurred by chance.

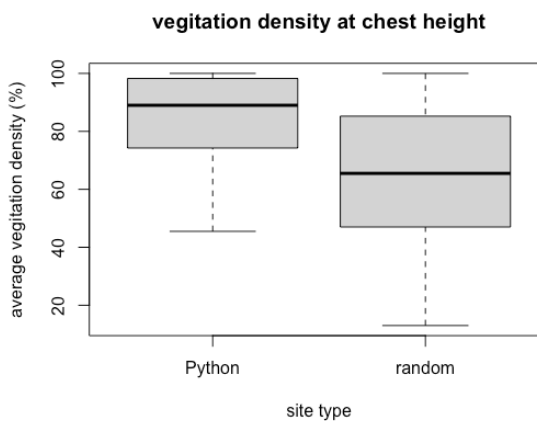
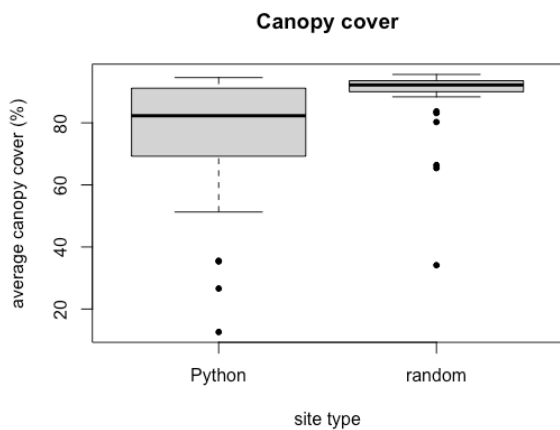


Figure. 2 A graph created in R studio showing the average canopy cover of the sleeping sites (labelled python) and the random sites. The graph shows that random sites had a higher average canopy cover of 90% and the sleeping sites had a lower canopy cover of 80% but also a larger interquartile range and lower outliers.

Figure. 3 A graph created using R Studio showing the average vegetation density at chest height. It shows that the sleeping sites (labelled python) on average had a higher percentage of vegetation density at 90% and the random sites on average had a vegetation density at chest height of 65%, neither categories have outliers and the random sites have a larger interquartile range.

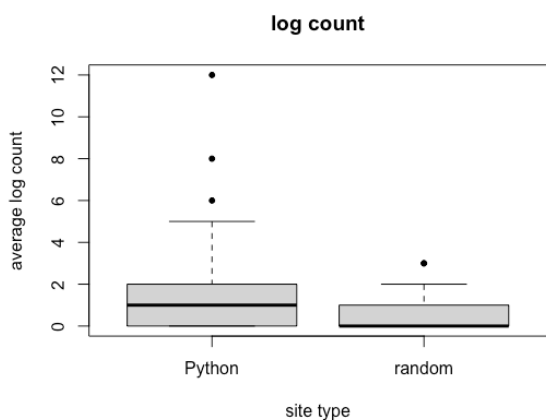


Figure. 4 A graph created using R studio showing the average log count for the sleeping (labelled python) and random sites. The graph shows that on average the sleeping sites had one log present and the random sites had zero. Both categories have outliers with the sleeping sites having the highest outlier of 12 logs and the random sites only three. The random sites only have an upper quartile range.

Figure 2 shows that the mean canopy cover for sleeping sites was 80% whereas the mean canopy cover for random sites was 90%, The sleeping sites show a much larger interquartile range than the random sites which have a much smaller interquartile range that overlaps with the sleeping sites upper quartile, they both have low outliers. The sleeping sites show a much larger range than the random sites. As shown in figure 3 the vegetation density at chest height at sleeping sites was 90% with a much lower 70% in random sites, suggesting they prefer much denser areas, the interquartile range for the sleeping sites is smaller than the random sites and there are no outliers, this data strongly supports a selection for highly dense areas takes place. Although the binomial GLM shows that there was a significant association between sleeping sites and log number.

Figure 4 shows that the mean number of logs at a sleeping sites is only 1 with a relatively small

interquartile range, the mean number of logs at a random site is zero with a small upper quartile range.

Generalised linear mixed model and prediction data frame

The results of the generalised linear mixed model showed the addition of a random variable (the individual pythons name) gave no explanation of the variation between site type, we were unable to run the model with all of the explanatory variables as there were not enough rows of data. This meant we were unable to distinguish if individual preference had masked any previously insignificant variables. We were also unable to produce a successful prediction data frame due to our low number of sampled sites, any predictions made from our data would have been unreliable.

Discussion

The findings of our study suggested that sites within the LKWS with higher levels of vegetation density at chest height and a higher log count, but a lower level of canopy cover had a higher probability of being a sleeping site. In our research we aimed to distinguish if reticulated pythons were actively selecting their sleeping sites or if this selection was random, we also aimed to characterise sleeping sites if a preference was detected. Despite the small sample size and some research limitations (see below), it appeared that pythons do actively select their sleeping sites as opposed to a random selection taking place. We identified that pythons appeared to select for environmental factors that predominantly provided coverage but also preferred a lower level of canopy cover than what is typically found within the forest.

While we were measuring log count, sleeping sites on average would only have one log present so the python could have been selecting for log presence rather than log abundance, this would make sense if the python is using the log for cover as if this is the case the presence of multiple logs would not be necessary. The high level of vegetation density detected also supports the theory that they are selecting for cover as it would be hard to spot the python amongst high levels of vegetation density. Reticulated pythons are nocturnal and would therefore be asleep during a time when the rest of the forest would be active. It is therefore likely that the pythons are selecting for camouflage and cover as a form of protection while they are sleeping, which is supported by the fact that they are very difficult to spot in the wild during the daytime (Natusch *et al* 2015). This would also protect them from predation when they are the most vulnerable. A similar study into the sleeping sites of the blue-lipped lizard (*Tiliqua scinidae*) in tropical Australia found a preference for canopy coverage (Price-Rees *et al.* 2013). While canopy cover was lower in sleeping sites than it was in random sites, this does not necessarily mean that reticulated pythons were not actively selecting for cover. At the sites with the lowest canopy cover there was still a high percentage of vegetation present at chest height. While canopy cover (which was measured from a human's perspective just above head height) was low, the reticulated python would still have been provided

cover by the vegetation density. This confirms our hypothesis that reticulated pythons are actively selecting sleeping sites, with environmental factors providing coverage as the selection criteria. However there appear to be very few specific requirements in this selection which could prove to be very beneficial for reticulated pythons if further fragmentation occurs in the LKWS.

Out of all of the parameters deemed insignificant vegetation density at ankle height was the most surprising, as vegetation at chest height appears to be a very important selection criteria. This could be explained by our research location. Our study took place in secondary forest where the majority of the forest floor was covered in vegetation due to there being less canopy cover in comparison to primary forest (Benitez-Malvido *et al* 2015). This meant there was little difference between the vegetation density at ankle height in sleeping sites and random sites, however this may not be the case in primary forest so further sleeping site analysis in other habitats could help to clarify if the findings of this study are limited to secondary forest.

Critical analysis of experimental design

Despite our small sample size, the finding that reticulated pythons are actively selecting their sleeping sites for cover appears to be a genuine result. However on top of our small sample size our research is also limited by the following assumptions.

- 1) This paper assumed that no untagged pythons had used the random sites as sleeping sites.
- 2) It also assumed that reticulated pythons use one sleeping site per day and do not relocate later on in the day. This assumption is bound to the limitations of VHF tracking as only one data point per animal per day is collected (Kays *et al.* 2011).
- 3) Tagging pythons with a VHF device is challenging and expensive. Therefore, only few animals were tagged and tracked. GPS tagging of pythons had been proved near impossible due to the battery requirement and the evident challenges of locating a python large enough to tag.
- 4) Age of the sleeping site. Sampling took place between one to four years after the pythons sleeping sites had been identified, this meant that some sites closer to the river had to be excluded due to erosion of the Kinabatangan river bank. Time in between tracking and sampling limits the validity of the study as over the years these sleeping sites could have changed drastically. What we recorded as a log could have been a tree at the time the python was using the site, the vegetation would have grown in that time and as the sites were close to the river they could have been flooded multiple times therefore changing the area before we analysed it.
- 5) GPS error: While the distance from sleeping sites to the Kinabatangan river did not end up being a significant variable, these results were not as accurate as they would have been closer to the date of tracking. These measurements also would have included error from the GPS points (around 5 metres) which would have also affected our accuracy in

relocating the python sleeping sites. This study would need to be compared with a study of more recent sleeping sites to see if similar results are observed, which would also give us a good idea of an expiration date for sleeping sites being analysed.

6) Data recording: vegetation density was recorded at chest height and ankle height.

However, the field assistant holding the density stick would change from one sampling day to another, leading to variation in the height the stick was being held at for reference. In the future, it would be better if the exact same person was holding the density stick across all of the survey sites or a designated height was decided for the stick to be held at. The insignificance of vegetation density at ankle height could also be due to a methodological flaw, 10 metres may have been too far away to have measured the vegetation density at ankle height. This could have been why there was no significant variation between sleeping sites and random sites as most readings of ankle vegetation density came to 100%. So further studies could consider either recording vegetation density at ankle height at a closer distance or using a different method to measure the vegetation density other than a density stick.

Application to future research

The reticulated pythons appeared to have environmental preferences for their sleeping sites but have shown to be adaptable to these site requirements. This adaptability means that their continued survival in a fragmented landscape is much more likely compared to other Bornean specialist snakes such as the broad headed snake (*Hoplocephalus bungaroides*) (Chandler *et al* 1990). We therefore assumed that continued habitat degradation will likely continue to not have a detrimental effect on the Reticulated pythons population at least not in terms of its sleeping sites. Future threats to the species will not come from them not being able to adapt to their habitat which further supports their claim as a generalists species, however further analysis into how their prey are affected by the habitat loss should be taken into consideration before truly declaring them as safe (Heard *et al* 2013). Having less sleeping site requirements could also mean they are much easier to translocate compared to other reptile species with greater sleeping site requirements such as the Telfairs skink (*Leiolopisma telfairii*) (Pernetta *et al* 2005), which could be useful in removing them from urban areas in cases of human/python conflict or other dangerous areas.

Conclusion and future research ideas

While we were unable to create a reliable prediction model with the limited data we had collected this could be reattempted with a larger data set. Prediction models can be great for predicting species presence (Hirzel *et al.* 2006) which would be useful in future research on the elusive species, either in attempting to tag the species or to determine its population size. However, it should be considered if further research continues to show that reticulated pythons have few sleeping site requirements, a reliable prediction model might not be possible as there might not be

enough variables to accurately narrow down a python sleeping site. Sites with high vegetation levels, lower canopy cover and logs can be found all over the tropical rainforest and may then not always mean that a python can be found sleeping there.

The results seem to suggest that Reticulated pythons prefer a site with a log for cover. Future work could be looking at the length, width and diameter of the log, presence of hollows, measurements of the hollow and possibly tree species preference as previous species have lost crucial habitat with the loss of dipterocarps due to both legal and illegal logging (Maycock 2012). Our research also strongly suggested that high levels of chest height vegetation density are important in sleeping site selection. Therefore, further analysis into the species of these plants could help to highlight if the pythons are selecting for the vegetation or if they are simply selecting for any type of cover. The results of our binomial GLM lacked confidence however a larger sample size would help to clarify the selection that took place. A larger sample size would also allow for a comparison between sleeping site selection in males and females and if body size has any effect on selection, as it has previously been found to effect habitat selection in Boid snakes (*Epicrates monensis*) (Chandler *et al.* 1990). There were also cases of individual pythons spending multiple days at sleeping sites with one individual spending two week at one sleeping site, further models could look into what makes these sites particularly preferable.

Further studies could also include a comparison with sleeping sites in primary forest, secondary forest, plantations and urban areas to see if the pythons are selecting for the same parameters or if they have adapted to their habitats differently. This could also help to clarify if they are definitely selecting for these parameters or just for cover in general. Reticulated pythons appear to thrive in the fragmented landscape of the Lower Kinabatangan Floodplain where other species, such as the flat headed cat (*Prionailurus planiceps*)(Evans *et al* 2016), are struggling to survive. To fully understand why this is the case further research into their sleeping site preferences should be paired with their home ranges and population size in the LKWS. While our first preliminary study into the sleeping site selection of the reticulated python confirms that pythons are actively selecting sleeping sites, and highlights what they could be selecting for, there is still a long way to go before we fully understand the reticulated pythons ecology and its conservation application.

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Supporting information

Figure.5 A table showing the raw data collected with all the variables measured for all 72 sites analysed.

54.0063 2787	56.8629 3914	57.7349 5192	57.1778 4289	33.9916 9242	45.4867 1597	40.8259 2482
304.24	353.3	677.99	591.11	576.23	608.88	555.24
499.25	671.25	240.75	509.25	611	950.75	497.75
present	present	present	present	present	absent	present
3	1	3	0	1	0	2
7	13	14	8	7	0	6
1	1	4	2	3	1	2
100	92	95.5	100	59	77	86.5
100	100	95	100	74.5	97	70
83.69	59.412	92.829	71.551	95.05	26.611	89.421
Cahaya	Cahaya	Cahaya	Cahaya	cahaya	Cahaya	Cahaya
random	Python	random	Python	random	Python	random