



TROPICAL BIOLOGY ASSOCIATION

Field Course Project Reports

Borneo

1st – 28th October 2018

The Tropical Biology Association is an NGO that is launching careers of conservation scientists and professionals from around the world. TBA's field courses are unique because they bring together students and teachers from the host region and internationally in equal numbers. Around 20 nationalities take part on each course, creating a dynamic forum for sharing ideas and developing networks that will last well into the future.

During the TBA field courses, students design and carry out their own projects, which are analysed and written up during the last 12 days of the course. For many, this is the first opportunity that students have had to carry out their own, original research. Many TBA projects have led to students' first publications (at least 36 papers have been published from the TBA students' course projects).

We are delighted to share the students' projects from the 2018 TBA course in Borneo.

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Edge effects and insectivorous birds in Danum Valley, Borneo

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Abstract

Forest edges differ to the interior core in terms of microclimate, level of disturbance and vegetation structure which can consequently alter faunal species assemblages. Even clearance for roads where one forest edge is nearby to another edge (i.e. roads) is sufficient to have negative consequences on their abundances and distributions. Insectivorous birds are particularly sensitive to habitat disturbance since the typically warmer and drier conditions at the edge negatively affects their invertebrate diet. Abundance estimates obtained from distance sampling were inconclusive due to large confidence intervals.

INTRODUCTION

Deforestation resulting from land use change for infrastructure poses a major threat to tropical biodiversity, by increasing the amount of edge per unit land area and creating further fragmentation. This presents a serious challenge in tropical rainforests, especially in South East Asia, which harbour the largest proportion of global biodiversity yet are anticipated to face substantial increases of infrastructure development (Alamgir *et al.*, 2017). Forest edges differ from the interior core in terms of microclimate, level of disturbance and vegetation structure which can consequently alter faunal species assemblages (Laurance, 2000). Various ecological parameters have been observed to be affected differentially with increasing distance from edge to the interior. For example, canopy and sub-canopy damage has been reported up to 150 m from edges of tropical forests due to exposure from clearance subjecting newly-established edges to extreme climatic effects (Lovejoy, 1983). Edge effects can therefore be an issue for core-adapted species that depend on dense vegetation and lower relative levels of disturbance which may become restricted to smaller areas of suitable habitat following clearance.

Various tropical avian species are strongly influenced by disturbance and fragmentation (Brooks & Balmford, 1996; Cleary *et al.*, 2007), however a detailed understanding of how forest edges influence their population densities is lacking. In particular, information concerning these impacts on insectivorous birds is scarce, which is surprising considering their prevalence may indicate forest productivity and health (Zakaria *et al.*, 2005). Furthermore, insectivorous birds are particularly sensitive to habitat disturbance (Fimbel *et al.*, 2001) since the typically warmer and drier conditions at the edge negatively affects their invertebrate diet (Zanette *et al.*, 2000). For some insectivorous tropical bird species, even clearance for roads where one forest edge is nearby to another edge (i.e. roads) is sufficient to have negative consequences on their abundances and distributions (Brooks & Balmford, 1996). This may be attributed to their lack of behavioural and

physiological capacity to cross gaps meaning populations within fragments will become isolated. With projections indicating a significant increase in construction of paved roads in the coming decade in South East Asia, it is fundamental that the impacts of gaps in vegetation on bird species are examined to allow for effective forest management and conservation of species.

Studies examining disturbance and bird species inhabiting lowland tropical forest may not accurately assess the consequences of this since the densely vegetated habitat makes identifying species and accurately counting them a challenge. We experimented with playback calls to assess the potential to overcome such sampling difficulties arising from tropical forests comprised of dense understorey. We selected four focal species that are known to both respond well to these calls and occur in relative high abundance in the study area.

AIMS

The aims were to investigate:

- i. the effects of forest edge on insectivorous bird species abundances at increasing distances from the edge to the interior,
- ii. whether vegetation density affects bird densities.

We hypothesised that densities of the insectivorous bird species will increase along a gradient from the forest edge to core interior. We also hypothesised vegetation density will have a positive effect on bird densities.

MATERIALS AND METHODS

Study site and species

Fieldwork was conducted in primary forest at Danum Valley in Sabah, northern Borneo (c. 04°57'45.33" N and 111°48'09.79 E). Paired insectivorous species Rufous-crowned Babbler *Malacopteron magnum*, Scaly-crowned Babbler *Malacopteron cinereum*, Black-and-yellow Broadbill *Eurylaimus ochromalus* and Banded Broadbill *Eurylaimus javanicus* were surveyed since they are common residents, territorial and known to respond well to playback calls.

Bird surveys

Birds were surveyed using a distance sampling point count technique (Buckland *et al.*, 2001) between 18th and 23rd October 2018 from 06:00 to 12:00 (noon). Point counts were completed by two observers every 75 m along five transects from the forest edge to the interior and positioned at least 100 m apart to minimise the risk of counting the same individual more than once (Figure 1). Transects were revisited once in reverse order on following visits to avoid time-of-day biases. The forest edge at rivers measuring over 30 m wide, determined using a laser range finder, were used as a proxy for road edges since both lack terrestrial

vegetation. Playback calls (Xeno-Canto, 2018) were used to survey birds for five minutes at each point for each species to allow a sufficient sample size to be recorded with minimal effort. The order in which calls were played was randomised at every transect. The bearing and distance from the researcher to responding birds were estimated using distance bins of 0-12.5 m, 12.6-25 m and 26-37.5 m.

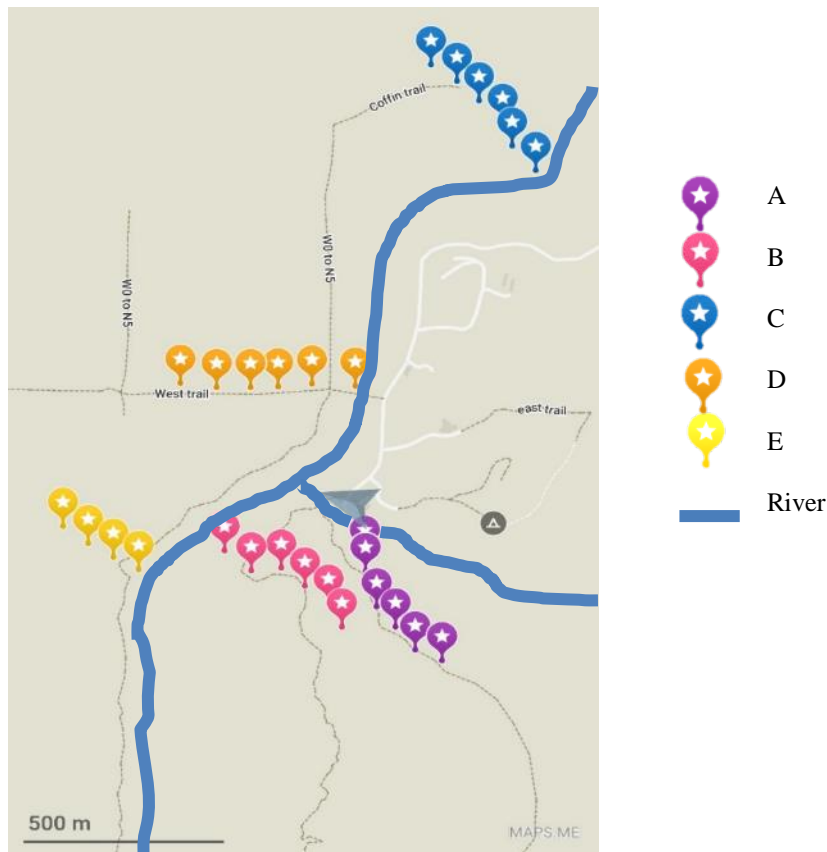


Figure 1. Map showing distributions of point transects from forest edge to interior within Danum Valley.

Habitat factors

To assess the influence of vegetation composition on bird densities canopy cover was used as a proxy and was recorded using a densiometer at four points 5 m away from the observer in a north, east, south and west direction.

Data analysis

Detection function models were applied to the species distance sampling data in Rstudio (R Core Team, 2017) package *Distance* (Miller, 2017). The model's goodness of fit was then assessed. Non-significant goodness of fit test statistics indicated a plausible detection model. When several plausible detection function models were produced, the AIC (Akaike's Information Criterion) were applied to select the best model. The most parsimonious model for every species was selected as Delta AIC values were less than 2 every time. The *summary* function within package *Distance* (Miller, 2017) provided the abundance and density

estimates for the four species. Linear regressions were then used to assess the relationship between species abundance estimates as a function of increasing distance from edge and canopy cover. Model assumptions were then validated using plots to assess for homogeneity, residual normality and influential observations.

RESULTS

Distance sampling abundance estimates for each point region (i.e. 0 m, 75 m, 150 m, 225 m, 300 m, 375 m) were inconclusive for all species due to very large confidence intervals (Table 1). Abundance estimates for all species did not differ with increasing distance from the edge (Figure 2). Furthermore, there was no effect of canopy cover on bird abundances: Scaly-crowned Babbler ($R^2 = 0.3222$, $F_{3,2} = 0.3169$, $P = 0.8171$); Rufous-crowned Babbler ($R^2 = 0.8472$, $F_{3,2} = 3.6980$, $P = 0.2201$); Black-and-yellow ($R^2 = 0.8264$, $F_{3,2} = 3.173$, $P = 0.2488$) and Banded Broadbill ($R^2 = 0.2412$, $F_{3,2} = 0.2119$, $P = 0.8816$).

Table 1. Distance sampling abundance estimates for four insectivorous bird species from forest edge to the interior

Species	Covered Area	Number of Transects	Distance	Canopy Cov%	Abundance Estimate	SE	LCL	UCL
Scaly-crowned Babbler	45364	5	0	85.4764	4.175	4.279	0.431	40.366
	45364	5	75	93.058	16.701	8.665	5.05	55.231
	45364	5	150	91.8776	4.175	4.279	0.431	40.366
	31755	5	225	92.7	23.859	17.192	3.712	153.34
	36291	4	300	94.754	15.756	9.761	3.216	77.183
	36291	4	375	94.748	7.878	4.88	1.608	38.591
Rufous-crowned Babbler	36291	5	0	85.4764	1.869	1.964	0.155	22.527
	45364	5	75	93.058	5.982	3.396	1.775	20.158
	45364	5	150	91.8776	5.982	4.766	1.048	34.14
	40828	5	225	92.7	4.985	2.383	1.84	13.507
	36291	4	300	94.754	1.411	1.482	0.117	17.002
	36291	4	375	94.748	2.822	1.865	0.603	13.205
Black-and-yellow Broadbill	45364	5	0	85.4764	2.357	1.352	0.702	7.912
	45364	5	75	93.058	4.125	2.911	0.897	18.959
	45364	5	150	91.8776	3.536	2.081	1.015	12.31
	40828	5	225	92.7	3.93	1.774	1.578	9.78
	36291	4	300	94.754	3.336	1.28	1.559	7.139
	36291	4	375	94.748	3.892	2.297	1.037	14.602
Banded Broadbill	45364	5	0	85.4764	0	0	0	0
	45364	5	75	93.058	0.397	35.806	0.0001 7077	0.00019
	45364	5	150	91.8776	0	0	0	0
	40828	5	225	92.7	0	0	0	0
	36291	4	300	94.754	0.156	20.293	0.0001 0673	0.00012
	36291	4	375	94.748	0	0	0	0

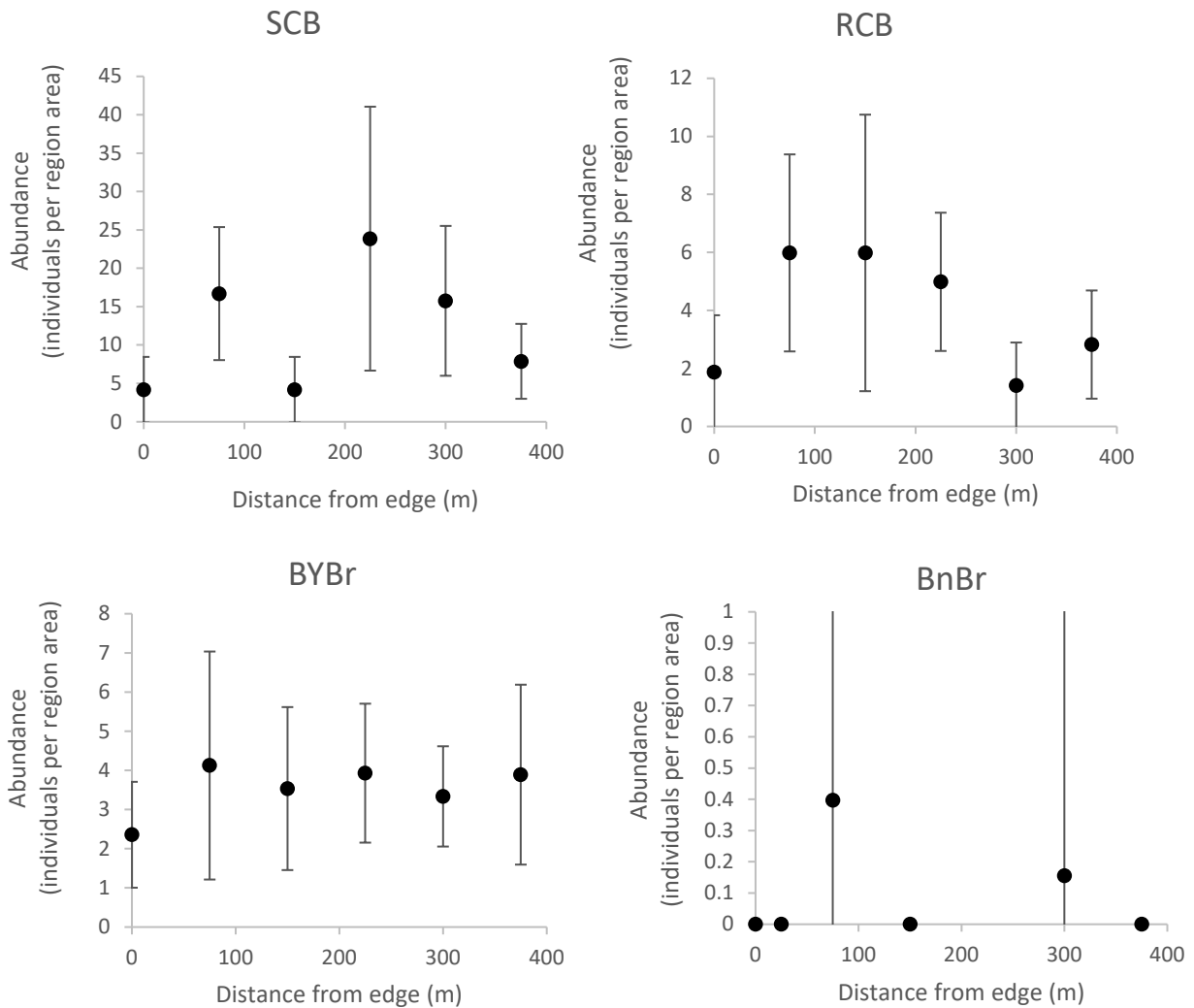


Figure 2. Abundances of four bird species from forest edge into the interior in primary forest at Danum Valley. SCB = Scaly-crowned Babbler, RCB = Rufous-crowned Babbler, BYBr = Black-and-yellow Broadbill, BnBr = Banded Broadbill. NB: Error bars were too large to include for BnBr.

DISCUSSION

Although abundances for Scaly-crowned Babbler and Rufous-crowned Babbler appear to increase threefold in number from the edge point to the 75 m point region, it is not possible to confidently infer much about the abundance estimates of the four study bird species in this study since there were significantly large confidence intervals. This was likely due to the low sampling effort, arising from the small sample size. Achieving a higher sampling effort by increasing the length and total number of transects may have provided sufficiently small abundance confidence intervals.

Using a river as a proxy for roads perhaps also gave false negative results since the edge types differ, whereby river edges will provide higher humidity and less variable temperature. Future studies should examine the effects of actual roads where possible, to allow for a more accurate representation of how their construction could alter avian species assemblages. This should be accompanied by taking measurements

of environmental factors that this study did not account for, such as temperature, humidity, light intensity and quantification of vegetation densities and types (Hosseini *et al.*, 2009) as using canopy cover alone as a proxy for vegetation structure failed to account for the high variability of understorey cover between sampling points.

Future work in this field could also be carried out in the logged forest of Danum Valley since primary forest in Borneo is now under a high level of protection, whereas there is high demand for logged forest to be sold to oil palm producers. It may therefore be more relevant to focus on logged forests to conserve a greater number of species.

Unexpectedly, The Black-and-yellow and closely related Banded Broadbill were never observed to co-occur at any point suggesting that they may compete for the same resources and establish and maintain mutually exclusive territories to avoid competition. In fact, the Black-and-yellow Broadbills often quickly responded to Banded Broadbill playback calls but not vice-versa. There are three possible explanations for this: (1) Banded Broadbills are not as responsive to calls than Black-and-yellow Broadbills than previously thought and may have caused results to underestimate Banded Broadbill presence; (2) the Black-and-yellow Broadbills may be the responsible species of the pair that maintains territorial exclusivity and are thus very responsive to Banded Broadbill calls; (3) the sampling transects may be placed within sites that are predominated by Black-and-yellow Broadbills that are responsive to Banded Broadbill calls. Had the reverse situation occurred, and the transects were placed in sites predominated by Banded Broadbills, it is possible that Banded Broadbills would be very responsive to Black-and-Yellow Broadbill playback calls. Increasing the number of transects could have accounted for this potential site variability and uncovered the underlying cause of the Broadbill response phenomenon.

CONCLUSION

Playback call methods using distance sampling techniques may be a promising approach to avian sampling in dense forests, however conclusions from the results of this study cannot be deduced confidently due to low sampling effort. Yet understanding edge effects is integral to improving our understanding of forest fragmentation, the consequences of continued land use change and protected area conservation planning therefore studies concerning this should of high priority.

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Factors affecting frog biomass in streams of Danum Valley

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Abstract

Anurans play a vital role in the transfer of nutrients throughout the ecosystem, making them suitable models for ecological studies. Limited studies however, have been done on frog biomass with the current study being the first for Danum Valley. This study aims to investigate how frog diversity, canopy cover, and slope may affect frog biomass in three of the streams in Danum Valley. The survey was done for six nights in Cabin stream, Kalison stream, and W6S5 stream. Weights were obtained for a total of 228 individuals, with *Limnonectes leporinus* (23.5%) contributing the most biomass. The most abundant species, however, was *Meristogenys orphocnemis* (31%). The absence of correlation between abundance and biomass in this study was likely because of the differences in body structure of various species. Canopy cover and slope, both of which affect habitat structure, were both found to influence both frog biomass and abundance. The highest abundance and total frog biomass were both obtained from W6S5 stream, which is relatively more heterogeneous than the other two streams.

INTRODUCTION

Frogs, like other amphibians, are sensitive to minute changes in the environment, which makes them valuable bioindicators of an ecosystem's status. Borneo has rich anuran diversity, with currently 183 species of frogs, 76% of which are found only on the island (Inger *et al.*, 2017). Such high diversity has been known to positively affect various ecosystem services (Oehri *et al.*, 2017).

Aquatic habitats are influenced by forest canopy cover which determines species distribution by limiting or altering the light availability and the litter input that affects decomposition process and primary productivity (Mokany *et al.*, 2008; Vannote *et al.*, 1980). It is an important factor in ecosystem processes and community structure shaping (Batzer, Palik & Buech, 2004; Vannote *et al.*, 1980). Previous studies on amphibians show that increasing canopy cover decreases amphibian abundance (Binckley & Resetarits, 2007; Halverson *et al.*, 2003), survival (Thurgate & Pechmann, 2007; Werner & Glennemeier, 1999), species richness (Skelly *et al.*, 2005; Werner *et al.*, 2007), development (Halverson *et al.*, 2003; Williams *et al.*, 2008) and growth (Schiesari, 2006; Skelly *et al.*, 2002).

Even though it has been assumed that amphibians play an important role in the transfer of energy from aquatic to terrestrial systems due to their dependence on both habitat types, the evidence for this is still limited (Gibbons *et al.*, 2006; Inger *et al.*, 2017; Semlitsch *et al.*, 2014; Vitt & Caldwell, 2014). This study aimed to fill this gap by investigating some factors that may affect frog community biomass in different

streams in Danum Valley. Specifically, species richness, abundance, canopy cover and slope were examined.

MATERIALS AND METHODS

Study area

The study was conducted in three streams of Danum Valley, East Sabah, Malaysia. Cabin Stream (4°59'16.94"N 117°54'19.48"E), and Kalison Stream (4°58'16.81"N 117°48'58.72"E) are located in a logged forests while W6S5 Stream (4°57'41.94"N 117°48'24.59"E) is a part of the West Trail located in a primary forest.

Stream survey and biomass determination

One 300 m transect was laid out along each stream, marked every ten metres. Night surveys were conducted along these transects from 17th October to 25th October 2018 between 18:00 and 23:00, with an average of three hours for each night. Four people took part in the survey each night, two for each side of the stream. Morphometrics such as snout-vent length (SVL) and weight were recorded for each individual captured during sampling, and distance from the edge of the stream and distance from the substrate surface were also measured. Species, sex and type of substrate were also noted. Every individual was toe-clipped according to the scheme by Jean-Marc Hero (1989) with unique simple codes for each survey night. Marked individuals were placed in separate plastic resealable bags and weighed using 10 g, 30 g, 100 g or 1000 g Pesola bars depending on estimated weight. The bags previously containing frogs were also weighed after each release. The frogs' weights were calculated by subtracting the weight of the bag from the weight of the bag containing a frog.

Other stream characteristics were also noted, including cloud cover, water pH and temperature. Canopy cover and slope were also measured every ten metres of the transect using a canopy densiometer and clinometer, respectively.

Statistical analysis

Regression analysis was done using basic pre-installed packages in R to find any correlation between different factors across the streams, including biomass & diversity (measured as species richness), biomass & canopy cover, and biomass & slope. Linear regression models of SVL and mass were also made for species with sample sizes of at least 10 individuals. These models were used to calculate the mass for individuals with only the SVL recorded. Total biomass per species was also plotted against the respective abundance to determine any correlation between these two factors.

RESULTS

A total of 228 individuals from 17 species and 7 anuran families were sampled in 3 streams for 6 sampling nights, amounting to a total frog biomass of 2,304.05 g. W6S5 stream had the highest frog biomass and abundance while Kalison had the highest species richness. Cabin stream, on the other hand, had the highest canopy cover. The highest mean slope and slope variance throughout the transect was also recorded for W6S5 (Table 1).

Table 1. Frog diversity, abundance, total frog biomass and characteristics of Kalison stream, Cabin stream, and W6S5 stream

Stream	No. of species	No. of individuals	Total frog biomass (g)	Canopy cover (%)	Slope	
					Mean	Variance
Kalison	12	66	603.2098	85.64	2.112903	1.678495
Cabin	9	42	684.8895	88.53	2.483871	1.458065
W6S5	11	120	1015.9488	87.16	4.048387	8.439247

The bulk of the biomass obtained from the streams was from *Limnonectes leporinus* (23.5%) while most of the frogs sampled belong to species *Meristogenys orphocnemis* (31%) (Table 2). Linear regression models of the mass and SVL was done for five species ($n \geq 10$) (Figure 1). No correlation was found between total biomass and abundance of each species (Figure 2).

Table 2. Biomass percentage and abundance percentage of various frog species sampled in Danum Valley streams, including individuals whose mass were obtained from regression models made for this study

Species	Abundance (%)	Biomass (%)
<i>Alcalus baluensis</i>	0.88	0.20
<i>Ansonia spinulifer</i>	3.95	1.40
<i>Chalcorana megalonesa</i>	14.04	7.82
<i>Chaperina fusca</i>	1.75	0.16
<i>Ingerophrynus divergens</i>	0.88	0.48
<i>Leptobrachium abbotti</i>	0.44	0.63
<i>Leptolalax sp.</i>	10.53	3.51
<i>Limnonectes finchi</i>	0.44	0.22
<i>Limnonectes ingeri</i>	0.88	7.32
<i>Limnonectes kuhlii</i>	13.16	20.42
<i>Limnonectes leporinus</i>	3.07	23.49
<i>Meristogenys orphocnemis</i>	31.14	19.90
<i>Phrynoidis juxtasper</i>	0.44	4.24
<i>Pulchrana picturata</i>	2.63	2.42
<i>Rhacophorus gauni</i>	0.88	0.27
<i>Rhacophorus pardalis</i>	1.32	0.66
<i>Staurois guttatus</i>	13.60	6.85

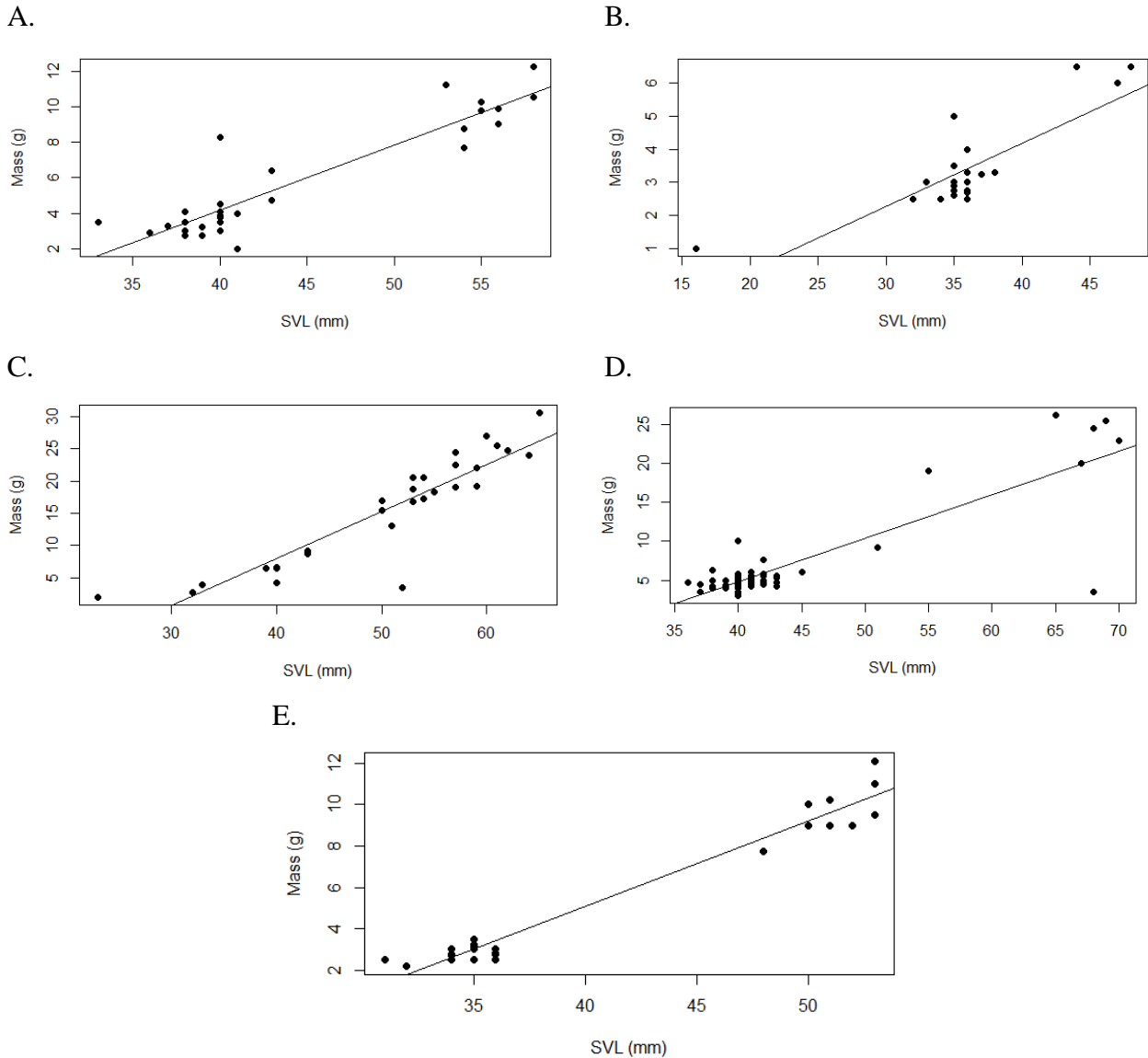


Figure 1. Linear regression models of SVL (mm) and mass (g) for some species sampled: A. *Chalcorana megalonesa*, ($F_{1,28} = 146.5$, $P = 1.21 \times 10^{-12}$), B. *Leptotalax* sp., ($F_{1,22} = 49.72$, $P = 4.489 \times 10^{-7}$), C. *Limnonectes kuhlii*, ($F_{1,44} = 126.9$, $P = 1.043 \times 10^{-11}$), D. *Meristogenys orphocnemis*, ($F_{1,66} = 186.5$, $P = 2.2 \times 10^{-16}$), E. *Staurois guttatus* ($F_{1,27} = 709.4$, $P = 2.2 \times 10^{-16}$).

The highest abundance (61 individuals) and total frog biomass (580.93 g) were obtained both at the W6S5 stream at different sampling nights. The first survey night at the Cabin stream returned the least individuals sampled (15), lowest total biomass (224.5 g) and lowest diversity (5 species) out of all the sampling nights. The highest number of species was recorded during the second sampling at the Kalison stream with 11 species. *Leptotalax* sp., *Limnonectes kuhlii*, *M. orphocnemis*, and *Staurois guttatus* were the most common species, found in all three streams and in every sampling night (Table 3).

Upon comparing results for the six sampling nights, linear regression analysis revealed no correlation between total frog biomass and species richness per sampling night ($F_{1,4} = 0.1251$, $P = 0.7414$) and

canopy cover and total frog biomass per stream ($F_{1,1} = 0.04903$, $P = 0.8613$). There was, however, positive correlation both between frog biomass and mean slope ($F_{1,1} = 2.442 \times 10^4$, $P = 0.004074$) and frog biomass and slope variance ($F_{1,1} = 20.82$, $P = 0.1373$) (Figures 5a and 5b).

Table 3. Diversity, abundance, and total biomass during the six sampling nights of the survey

Kalison Stream, 17 th October 2018			
No.	Species	Abundance	Biomass
1	<i>A. baluensis</i>	1	2
2	<i>A. spinulifer</i>	3	10.4
3	<i>C. megalonesa</i>	11	46.41525
4	<i>Leptolalax</i> sp.	6	20.8
5	<i>L. kuhlii</i>	2	34.75
6	<i>L. leporinus</i>	1	11
7	<i>M. orphocnemis</i>	3	14
8	<i>P. juxtasper</i>	1	97.75
9	<i>R. pardalis</i>	1	3.5
10	<i>S. guttatus</i>	2	12.2
TOTAL		31	252.81525

Cabin Stream, 18 th October 2018			
No.	Species	Abundance	Biomass
1	<i>Leptolalax</i> sp.	2	6.25
2	<i>L. kuhlii</i>	4	64
3	<i>L. leporinus</i>	1	73.5
4	<i>M. orphocnemis</i>	5	59.75
5	<i>S. guttatus</i>	3	21
TOTAL		15	224.5

W6S5 Stream, 20 th October 2018			
No.	Species	Abundance	Biomass
1	<i>C. megalonesa</i>	9	51.89869
2	<i>C. fusca</i>	4	3.7
3	<i>I.s divergens</i>	1	4
4	<i>Leptolalax</i> sp.	2	5.25
5	<i>L. kuhlii</i>	10	195.85449
6	<i>M. orphocnemis</i>	19	97.26209
7	<i>P. picturata</i>	3	33
8	<i>R. gauni</i>	1	1.75
9	<i>S. guttatus</i>	12	42.29878
TOTAL		61	435.01405

Kalison Steam, 23 rd October 2018			
No.	Species	Abundance	Biomass
1	<i>A. baluensis</i>	1	2.5
2	<i>A. spinulifer</i>	2	4.95
3	<i>C. megalonesa</i>	5	26.95
4	<i>Leptolalax</i> sp.	3	12.6
5	<i>L. finchi</i>	1	5.15
6	<i>L. kuhlii</i>	2	19.5
7	<i>L. leporinus</i>	3	188.75

8	<i>M. orphocnemis</i>	11	55.6
9	<i>R. gauni</i>	1	4.5
10	<i>R. pardalis</i>	2	11.6
11	<i>S. guttatus</i>	4	18.29452
TOTAL		35	350.39452

Cabin Stream, 24th October 2018

No.	Species	Abundance	Biomass
1	<i>C. megalonesa</i>	2	12.5
2	<i>L. abbotti</i>	1	14.5
3	<i>Leptotalax</i> sp.	2	9.25
4	<i>L. ingeri</i>	2	168.75
5	<i>L. kuhlii</i>	3	62.25
6	<i>L. leporinus</i>	1	83
7	<i>M. orphocnemis</i>	7	57.88945
8	<i>P. picturata</i>	3	22.75
9	<i>S. guttatus</i>	6	29.5
TOTAL		27	460.38945

W6S5 Stream, 25th October 2018

No.	Species	Abundance	Biomass
1	<i>A. spinulifer</i>	4	17
2	<i>C. megalonesa</i>	5	42.45
3	<i>I. divergens</i>	1	7
4	<i>Leptotalax</i> sp.	9	26.65
5	<i>L. kuhlii</i>	9	94.2
6	<i>L. leporinus</i>	1	185
7	<i>M. orphocnemis</i>	26	174.03473
8	<i>S. guttatus</i>	4	34.6
TOTAL		59	580.93473

DISCUSSION

Measuring faunal body conditions is a practical way of assessing habitat quality (Bancilla *et al.*, 2010). For amphibians, snout-vent-length (SVL) is the most commonly used morphometric (Vitt & Caldwell, 2014). Biomass, though, is what is used to measure the contribution of primary consumers to secondary production as it is a proxy for energy transfer from one trophic level to the next (Gibbons *et al.*, 2006; Townsend *et al.*, 2000). Some studies have employed a number of allometric models that have been proven to be robust enough to predict biomass from a given SVL with little error (Santini *et al.*, 2018). The same has been attempted for this study to increase the already limited sample size, though it could only be done for the most abundant species due to limited data points available for making the models. Nevertheless, using these models have increased the sample size by 3.5% and total biomass by 2.5%.

It is important to note, however, that SVL-mass models are affected by factors such as species biology, habitat condition, and individual condition (Santini *et al.*, 2018). Caution, thus, has to be applied when using them. Arboreal frogs are known to have lower SVL-mass ratio than terrestrial and semi-aquatic ones, which is believed to be limited by their locomotory mechanism. Increased mass may not allow tree frogs to land

on thin branches as they may break. Mass may also vary depending on sex and condition, such as if a female is gravid or not. For this study, however, all adults are pooled together per species due to limited time for analysis.

Due to these factors, total biomass per species per habitat does not necessarily correlate with the abundance of these species (Figure 4). The streams sampled were dominated by *M. orphocnemis*, a medium-sized, slender-bodied species with SVL ranging between 32-40 mm for males and 55-70 mm for females. However, it was *L. leporinus*, a large species with SVL ranging from 90-175 mm for males and 85-125 mm for females, which contributed the greatest to the total body mass for all the streams (Inger *et al.*, 2017). To further illustrate this difference: one individual of *Phrynoidis juxtasper* contributed 4.24% to the total biomass for all the streams, which is greater than the amount contributed by 24 individuals of *Leptotalax* sp.

Resource availability is also expected to affect SVL-mass ratios. With more resources, mass is expected to increase in greater increments per increase in SVL than in less favourable conditions. Diversity results in the utilisation of various types of niches, thus lifting competitive pressures on members of the community (Oehri *et al.*, 2017). A factor that may affect resources for frogs, especially during the tadpole stage, is canopy cover. Canopy cover was initially hypothesised to positively correlate to total biomass and diversity as canopy openness may provide frogs with less protection from predators beyond the canopy. Studies reveal, however, that by changing microhabitat conditions (i.e. lower temperature), higher canopy cover lowers the survival of tadpoles thus leading to lower overall adult frog biomass for the ecosystem (Schieri, 2006; Skelly *et al.*, 2003). Reduced light penetration to the understorey may also lower productivity, thereby decreasing the ability of the system to support more species and individuals. Results of the current study coincide with these observations: Cabin stream which had the highest canopy cover (88.53%) also had the lowest total diversity, biomass, and abundance.

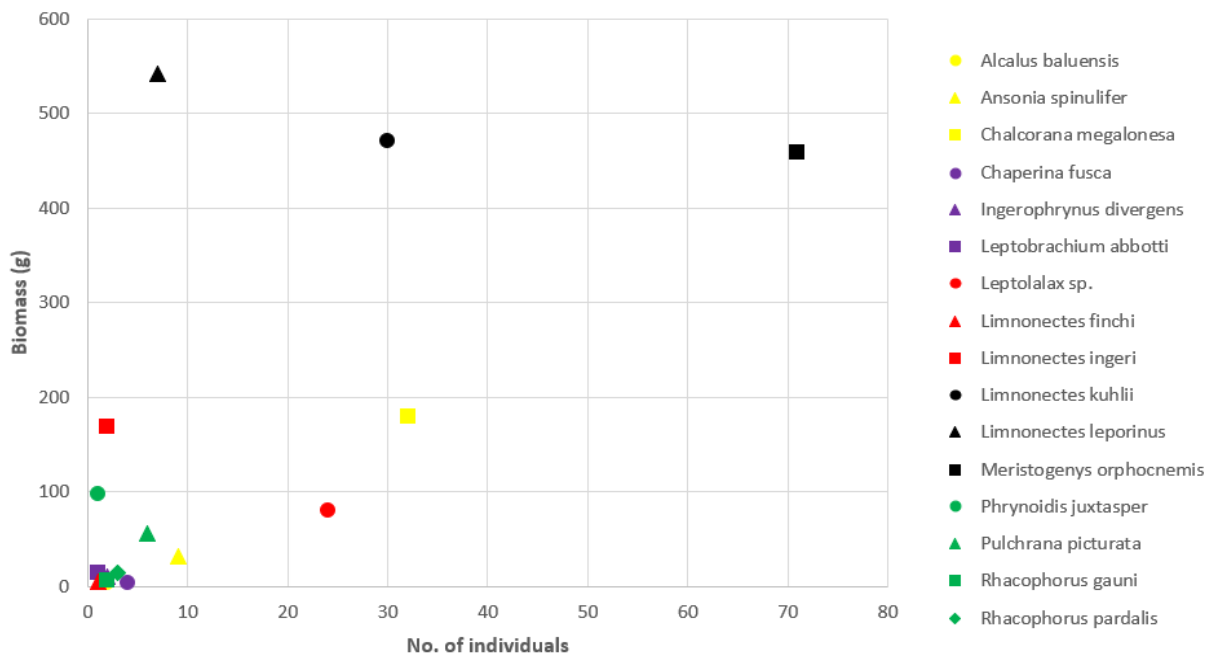


Figure 2. Abundance vs mass (g) of different frog species found in the Kalison, Cabin, and W6S5 streams of Danum Valley.

Habitat heterogeneity may also affect biomass and abundance. Slope was found to be positively correlated with frog biomass (Figure 3). W6S5, which had the highest total frog biomass and abundance for this study, also had the highest average slope (4.05°) and greatest slope variance (8.44) along the transect. Compared to Kalison and Cabin, it also had a more varied landscape with larger boulders and a large patch of land in the middle of the stream. All of these features may have provided more microhabitat types, more niches to be filled, that can support more types of individuals with diverse survival strategies.

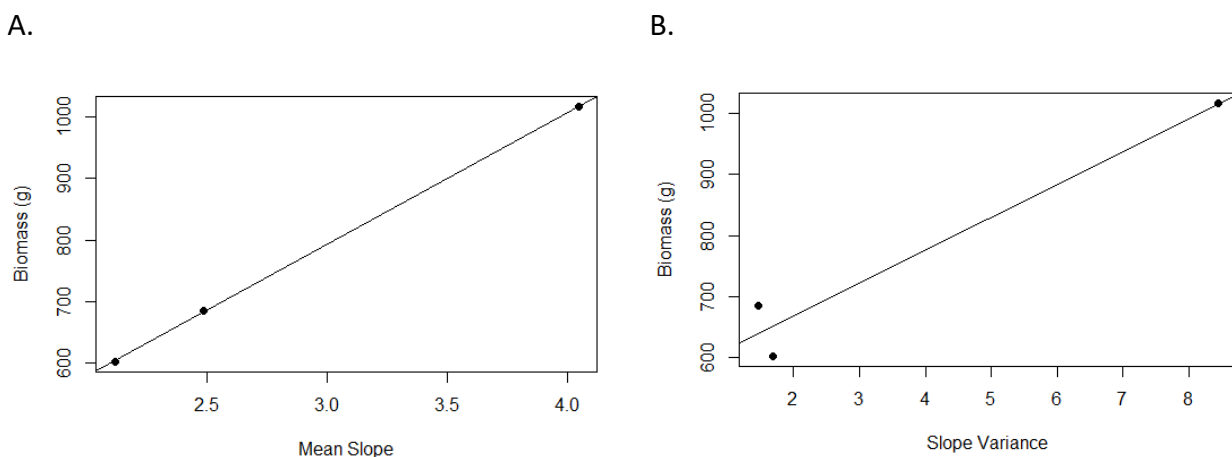


Figure 3. Linear regression models showing positive correlation between total frog biomass per stream and some stream characteristics. A. total frog biomass per stream and mean slope throughout the 300 m transect ($F_{1,1} = 2.442 \times 10^4$, $P = 0.004074$) and; B. total frog biomass per stream and slope variance throughout the 300 m transect ($F_{1,1} = 20.82$, $P = 0.1373$).

Biomass is an important measure of an ecosystem's condition by measuring biomass and comparing it with various ecological factors. This study, however, seems to lack sufficient data owing primarily to time constraints for doing the project. For similar studies in the future, it is thus recommended to increase the number of sampling sites or survey nights to increase sample size. Results from this project may also be evaluated against other vertebrates to illustrate and quantify energy transfers in different habitats in the area. A comparison of frog biomass change may also be done across different years. Such studies will be particularly beneficial because of Borneo's high anuran diversity and Danum Valley's status as a protected area.

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Home range and microhabitat preferences of *Limnonectes kuhlii* or “Needle in a haystack – never underestimate the work behind a paper”

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Insupp, University of Vienna, Austria and Natasha Woest, University of Salford,
United Kingdom**

Abstract

The purpose of this study is to gain knowledge about the home range of *Limnonectes kuhlii* and their microhabitat preferences in a dipterocarp rainforest in Sabah, Borneo. We used radio-tracking to get points of their locations during day and night and compared data of maximum distance travelled, distance to closest water bodies, their response to rainfall and the probability of being submerged. The longest distance travelled by one individual was 31.25 m. The distance separating a frog from its closest water body did not vary between midday and evening radio tracking events but declined in response to increasing rainfall. Frogs were also more likely to be buried or submerged during daylight.

INTRODUCTION

Due to forest conversion and landscape use, biodiversity may decrease rapidly. The impact of these large-scale land use changes is not yet fully understood and may further accelerate extinction rates (Gillespie *et al.*, 2012). Home ranges and habitat use varies tremendously amongst species, therefore it is necessary to quantify that diversity and understand the behaviours and ecologies governing distribution and abundance.

Amphibians are rapidly declining worldwide. According to the IUCN Red List (2018), 70% of the listed species of Sabah (Malaysia) are decreasing, or their trend is unknown, and 12.3% of the species endemic to Borneo are threatened with extinction. Tropical forest amphibians may have a low resilience to habitat disturbance and microclimatic changes. Reduced availability of forest debris for shelter and foraging, and loss of breeding microhabitats may lower their dispersal capabilities (Gillespie *et al.*, 2012). Despite extensive research into the distribution of frogs, further studies into their ecology, habitat use, and home range is beneficial for their conservation. Detailed information on species distributions are crucial for assessing their conservation status as listed on the International Union for the Conservation of Nature (IUCN) Red List of Threatened Species (Brito, 2010). Frogs have been known to migrate around a kilometre to their breeding sites (Inger *et al.*, 2017) yet the range, intensity and needs differ between species.

The dense primary forest at Danum Valley makes it an appealing habitat for anuran species with a network of rivers, streams and adequate rainfall. Frogs are highly dependent on water bodies for their breeding, feeding and desiccation avoidance. Therefore, the environment below the canopy cover provides different

niches suitable for studying this diverse group. A broad range of habitats are occupied by frogs found in Borneo and with the high annual rainfall, they are never far from a water source.

Home range and habitat use data is insufficient for *Limnonectes kuhlii*, a species of stream breeders which make up a proportion of the amphibian biomass. Radio-tracking is a technique used by scientists to track larger mammals, but transmitters are now available for smaller taxa such as amphibians. It is a useful tool determining habitat use, migratory connectivity and time spent in a particular habitat.

To get more information on their behaviour and habitat preferences radio tracking was used to address the following hypotheses:

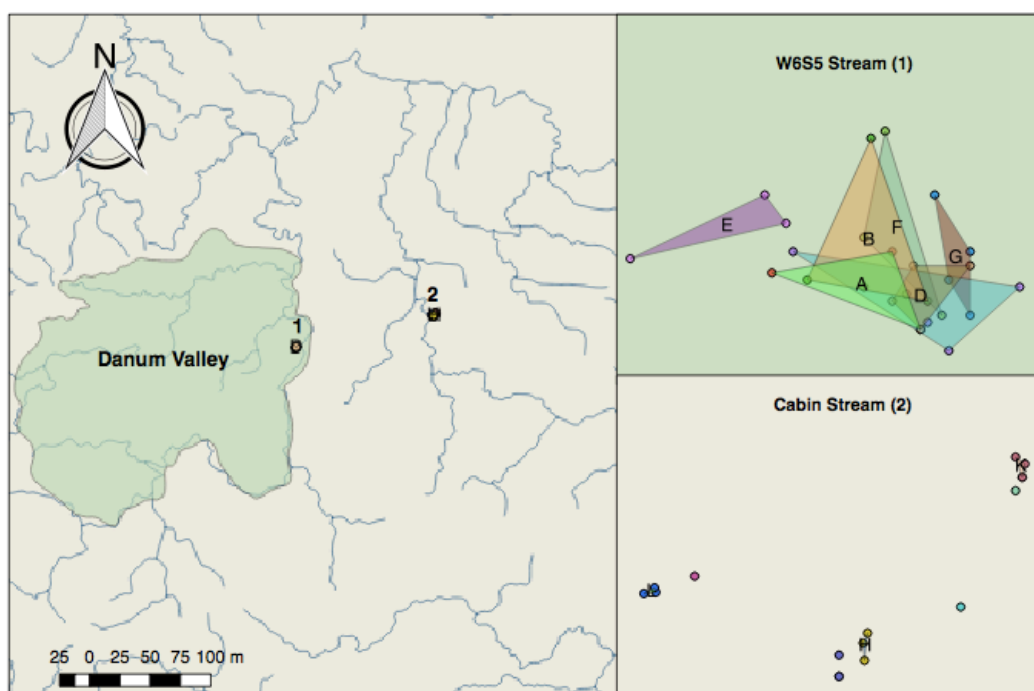
1. Time of day will influence the distribution and habitat use of the species.
2. *L. kuhlii* will move further away from water bodies during the day and stay closer to them at night.
3. The species will prefer a covered location during the day compared to the night.

METHODS

Study sites

This study was conducted at Danum Valley Field Study Centre (DVFSC) in Sabah, Malaysia.

Figure 1. Map of Danum Valley and the two study streams on the inset: W6S5 (1) and Cabin (2)



Danum Valley Field Study Centre comprises primary mixed Dipterocarp forest, surrounded by selectively logged forest, and receives rain year-round. Frogs were radio-tagged on two streams. One stream was located in primary forest (W6S5) and one in selectively logged forest (Cabin) (Figures 1 & 2).



Figure 2. Observed streams, A...W6S5, located in primary forest, B...Cabin, located in logged forest; Danum Valley, Sabah, Malaysia

Study species

Limnonectes kuhlii (Dicroglossidae) (Figure 3) were the selected species to radio track, a medium-sized frog which makes up between 10-30% of individuals found on stream surveys at Danum (Sheridan, unpublished data). Mature males have a snout-vent length (SVL) of 44–74 mm and females have an SVL of 51–67 mm. Sex is usually determined by the presence of long fangs in males and short fangs in females (Inger *et al.*, 2017), but because of considerable size overlap, and the presence of short fangs in immature males, it was not possible to confirm sex of each individual in the present study.

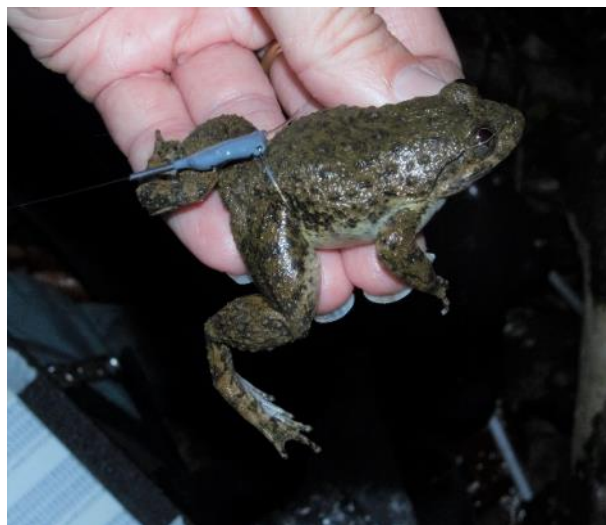


Figure 3. *Limnonectes kuhlii* showing transmitter and attachment belt

Data collection

Holohil BDX-2 transmitters, with a weight of 0.42 g and an estimated battery life of 12 d were attached to seven individuals on 16th October 2018 on W6S5, and to four individuals on the 18th October 2018 and two individuals on 20th October 2018 on Cabin. Due to the weight of transmitters plus attachment (0.4 g), we only tagged individuals with a body weight of > 9 g (transmitter less than 5% of body weight), or individuals with an SVL of >43 mm. To observe variation in movement patterns related to body size, we attempted to tag individuals of a range of sizes from 43–62 mm. Frogs were fitted with a transmitter following Sheridan *et al.* (2017) and released at the site of capture to minimise the stress implied by the procedure. Each initial location (site of release after tagging) was marked with a flag and its GPS coordinates were recorded. Frogs were radio-tracked from 16th–24th October 2018.

Frogs were tracked once daily using a portable radio receiver and a 3-element folding antenna for 2–9 d. Variation in number of days of tracking for each individual resulted from transmitters coming off of individuals (n = 2), battery failure and reduced signal range during rainfall (n = 20). Frogs were tracked during the day 09:00–15:00 h and at night 18:30–23:00 h, every day aside from 20 October when heavy rain prevented safe access to study sites. Frogs were defined as detected if they were visually encountered, or if the radio signal was at a strength of 10 when the receiver and antennae or only receiver was used when in close proximity.

When possible, located individuals were caught and visually inspected to ensure that they were unharmed by the transmitter attachment. The distance travelled by a frog since its last known location was measured as a straight line using a metre tape, and its compass bearing was recorded. A frog's vertical and horizontal distance to its closest water body was recorded each time it was located, including the initial frog capture event. If no signal was obtained within 500 m radius from the point where a frog was last located, radio tracking was abandoned until the next tracking event. Individual locations were entered into QGIS (2.18), and polygons were created for each individual to calculate total area traversed during the study.

Statistical analyses

The difference in mean frog habitat range perimeter among streams was tested using a linear model (LM), including the number of observations per individual as a covariate. The impact of time of day on microhabitat choice was tested by a general linear mixed-effects model (GLMM) involving a binomial distribution (1 = in refugia such as a hole in the ground or stream bank, or under leaf litter or under water, 0 = above ground and exposed, such as sitting on top of a rock at the stream's edge). To test whether time of day influences the distance between a sampled frog and their closest water body, a linear mixed-effects model (LMM) was used. The response variable was defined as the hypotenuse of their vertical and horizontal distance from the water.

In all mixed-effects models, daily rainfall was included as a covariate and frog ID, survey site, and date were included as random terms. Random terms with a variance <0.001 were removed. When creating maximal models, test statistics and p-values for non-significant terms were taken from the last model in which they occurred during the model simplification process. All statistical analyses were carried out in RStudio (RStudio Team, 2015), using the package lme4 (Bates *et al.*, 2015). Data on frog microhabitat use collected before radio transmitter attachment was excluded from the analyses to avoid detection bias.

RESULTS

Due to the immediate release of the frogs after the transmitter attachment, behavioural changes are uncertain. When individuals were located after the initial attachment date, none of them showed signs of rubbing or wounds caused by the transmitter attachment belt. Frogs were spotted visually or with the use of the transmitter 35% of the total survey times for both streams across midday and evening survey events.

The mean habitat range perimeter of a frog did not vary among survey streams (Figure S1; LM, 19.657 ± 11.648 , $F_{1,7} = 3.579$, $p = 0.100$).

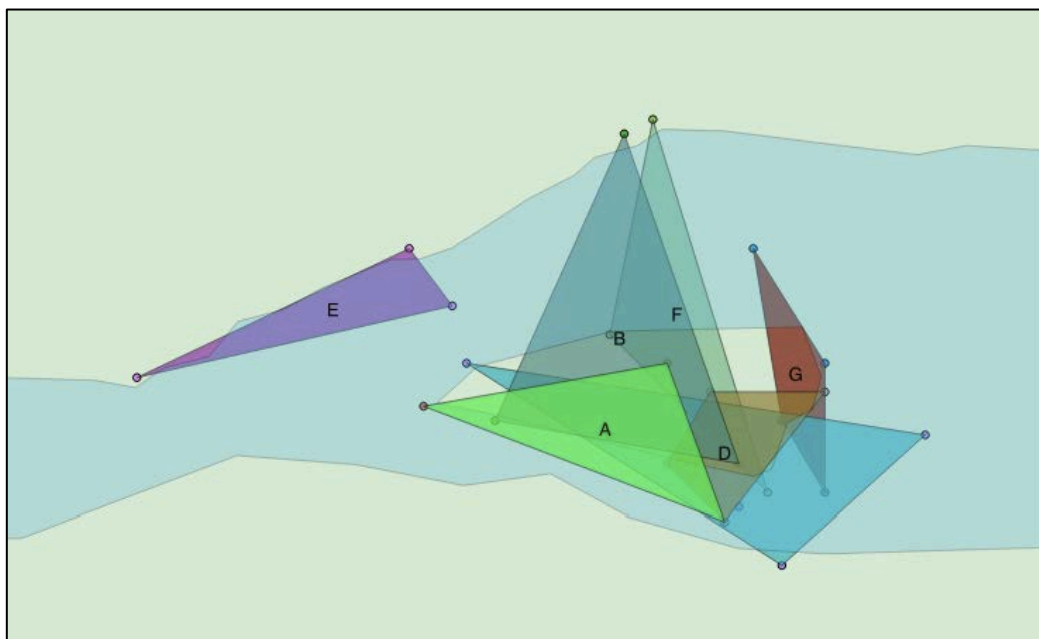


Figure 4. W6S5 Stream displaying the home range of each frog (A-G) with 3 or more detection points.

The distance separating a frog from its closest water body did not vary between midday and evening radio tracking events (Figure 1; Table Sx; LMM, -0.057 ± 0.220 , $\chi^2 = 2.614$, $p = 0.783$) but declined in response to increasing rainfall (Table Sx; LMM, 0.015 ± 0.008 , $\chi^2 = 3.875$, $p = 0.049$). Frogs were also 7% more likely to be buried or submerged during daylight (Table 2; mean midday probability = 1, mean evening probability = 0.136; GLMM, 59.751 ± 836.210 , $\chi^2 = 30.327$, $p = <0.001$).

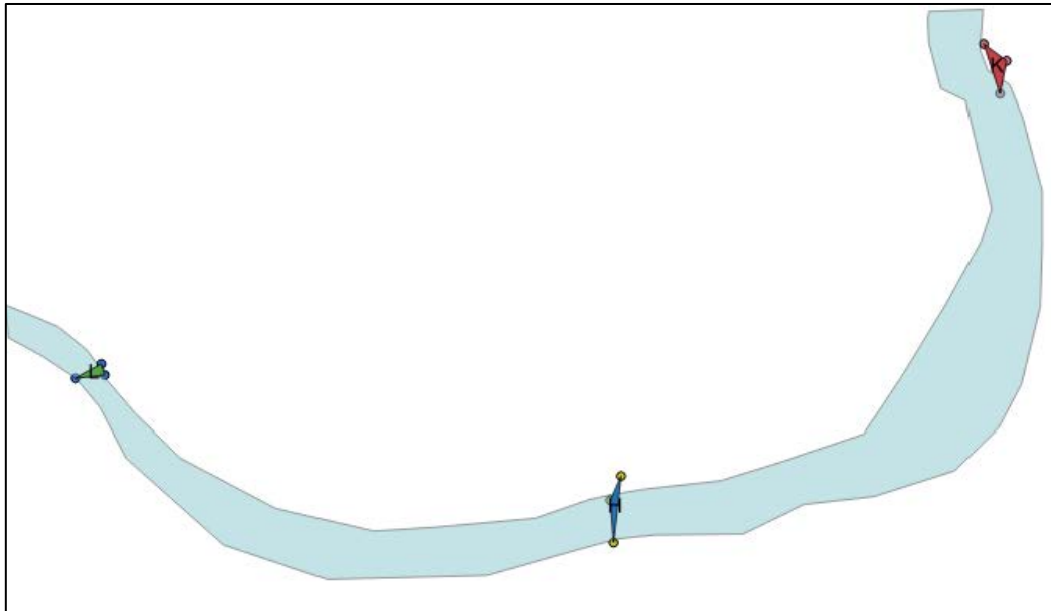


Figure 5. Cabin Stream displaying the home range of each frog (L, H, K) with three detection points.

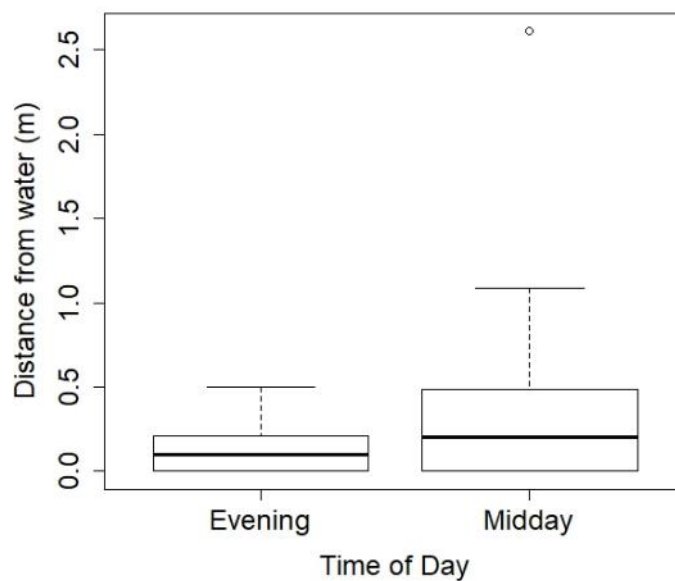


Figure 6. Comparison of a frog's distance to its closest water body among midday (boxes = median \pm 1Q, whiskers = values outside the IQR excluding outliers; sample: 28 observations).

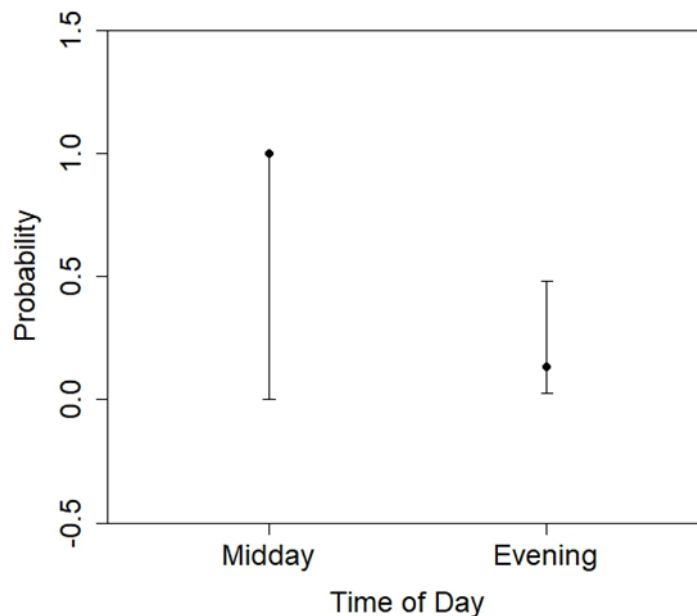


Figure 7. The probability of sampling a frog that is submerged or buried during midday and evening respectively, at average daily rainfall (points = group means, whiskers = standard errors; sample size: 28 observations within 2 streams).

DISCUSSION

The home ranges of *Limnonectes kuhlii* differed between the streams, where W6S5 had an average area covered of 57.09 m and Cabin was 21.76 m. Sample size ($n = 7$) at W6S5 was sufficient yet the data collection would have improved if the time period was longer. Frog B travelled the single longest distance of 31.25 m downstream whilst Frog F only travelled 7.7 m upstream. As the individuals were released in the close vicinity of the attachment site and not where they were originally located, it was interesting to note that they did not relocate to their original site of collection. This migration may occur, should the study had been longer therefore, it would be beneficial to consider the release site and distance travelled in relation to having a homing instinct or not. Not all the individuals were sexed due to uncertainty, but the average weight of individuals at this stream equated to 18.29 g with an average snout vent length (SVL) of 52.67 mm.

Cabin stream sample size was smaller ($n = 3$) as the attachment of the radio transmitters took place after W6S5, reducing the tracking days. Frog I travelled the longest distance, 17.52 m, whilst Frog K traversed the shortest distance, 6.3 m. The average area covered by the three individuals was 21.76 m. As the individuals were released at the site of initial finding, they did not cover a larger area as those at W6S5 from the number of observations ($F = 3.579$, $P = 0.100$, $DF = 1$). The average weight of the three individuals was 18.17 g with an average SVL of 60 mm.

Two previous studies have been conducted on frog radio-tracking in South East Asia, studying the movements of *Limnonectes leporinus* (n = 4) in Brunei (Grafe *et al.*, 2011) and *Meristogenys orphnocnemis* (n = 6) in Malaysia (Sheridan *et al.*, 2018) respectively. Both studies sampled ≤ 7 individuals which were sampled from one stream only and tracked over a longer period of time than in this study.

Frogs were more likely to burrow or submerge themselves in water during the day (Figure 4; Table S2). This apparent variation in microhabitat preferences may be caused by desiccation and/or predator avoidance during daylight hours when temperatures and frog conspicuousness is higher. A frog's distance from their closest water body was however unaffected by the time of day (Figure 5; Table S3), implying that their distance from a stream is independent of their microhabitat preferences. The negative rainfall effect on a frog's distance from water (Table S3) instead suggests that frog movements are limited by desiccation risk. To further explore this hypothesis, future studies should test the impact of canopy cover and humidity on frog movements. Our use of mean daily rainfall across day and night time surveys may limit the accuracy of our findings.

ACKNOWLEDGEMENTS

We thank Jennifer Sheridan for guiding us in the development of our project, providing the equipment and sharing her frog identification and radio tracking skills with us. Thanks also to research associates at the Danum Valley Field Centre for their invaluable help and assistance in the field. Thank you to Kevin Wallace for assisting with the acquisition of Field Assistants and equipment and always putting a smile on our tired faces. Finally, we thank the Borneo 2018 Tropical Field Course participants for their support and send a special thanks to Gabriel Hibberd, Amael Hinojo and Vincent Grognez for assisting during our night surveys and improving our field methods.

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APPENDIX

Table 1. Testing the predictors of a frog’s habitat range perimeter (m) (maximal model; sample size: 10 observations from 2 sites).

	Estimate	Std. Error	F value	Pr(>F)
<i>Intercept</i>	24.024	41.079		
<i>Stream</i>	19.657	11.648	3.579	0.100
<i>No. of observations</i>	4.081	13.345	0.094	0.769

Table 2. Predicting the distance separating a frog from their closest water body (maximal model; sample size: 28 observations from 2 sites).

	Estimate	Std. Error	Chisq.	Pr(<Chisq.)
<u>Fixed effects</u>				
<i>Intercept</i>	0.131	0.121		
<i>Daily rainfall (mm)</i>	0.015	0.008	3.875	0.049
<i>Time of day (Midday)</i>	-0.057	0.220	2.614	0.783
<u>Random terms</u>				
<i>Study stream</i>	0.002	0.002		
<i>Date</i>	0.003	0.003		

Table 3. The predictors of a frog’s probability of being submerged and/or covered during a survey event. Test statistics are given on the logit scale (maximal model; sample size: 28 observations from 2 sites)

	Estimate	Std. Error	Chisq.	Pr(<Chisq.)
<u>Fixed effects</u>				
<i>Intercept</i>	-11.933	6.485		
<i>Time of day (Midday)</i>	59.751	836.210	30.327	<0.001
<i>Daily rainfall (mm)</i>	-0.029	34.440	<0.001	0.973
<u>Random terms</u>				
<i>Frog ID</i>	2,205	46.96		

Table 4. Data of all streams and frogs captured for radio-tracking.

Stream	Frog ID	SVL (mm)	Weight (g)	No. of days tracked and observations	Maximum distance horizontal from stream	Maximum distance vertical from stream	Max distance (m)	Longest distance travelled between surveys (m)	Perimeter (m)
W6S5	A	52	18	6(3)	0	0.5	14.9	10	56.99
W6S5	B	NA	20	6(3)	0.2	0.1	47.19	31.25	70.34
W6S5	C	43	10	6(3)	0.5	0.5	11.73	10	34.21
W6S5	D	52	19.5	4(4)	0	0.5	22.81	14.91	79.71
W6S5	E	52	16.5	6(3)	1	0.43	33.68	26.2	53.85
W6S5	F	57	22	4(3)	2.55	0.55	12.26	7.7	64.23
W6S5	G	60	22	6(4)	0.2	0.47	35.26	23.3	40.3
Cabin	H	61	22.5	3(3)	0	0.2	17.08	9.2	46.08
Cabin	I	59	19.5	1(1)	0	0	17.52	17.52	0
Cabin	J	59	17.5	2(1)	0.1	0.82	11	11	0
Cabin	K	60	20	3(3)	0.1	0	12.26	6.3	38.64
Cabin	L	48	12	3(3)	0.2	0	15.39	7.3	24.08
Cabin	M	62	20.5	1(1)	-	-	-	-	-
Cabin	N	58	16	1(1)	-	-	-	-	-

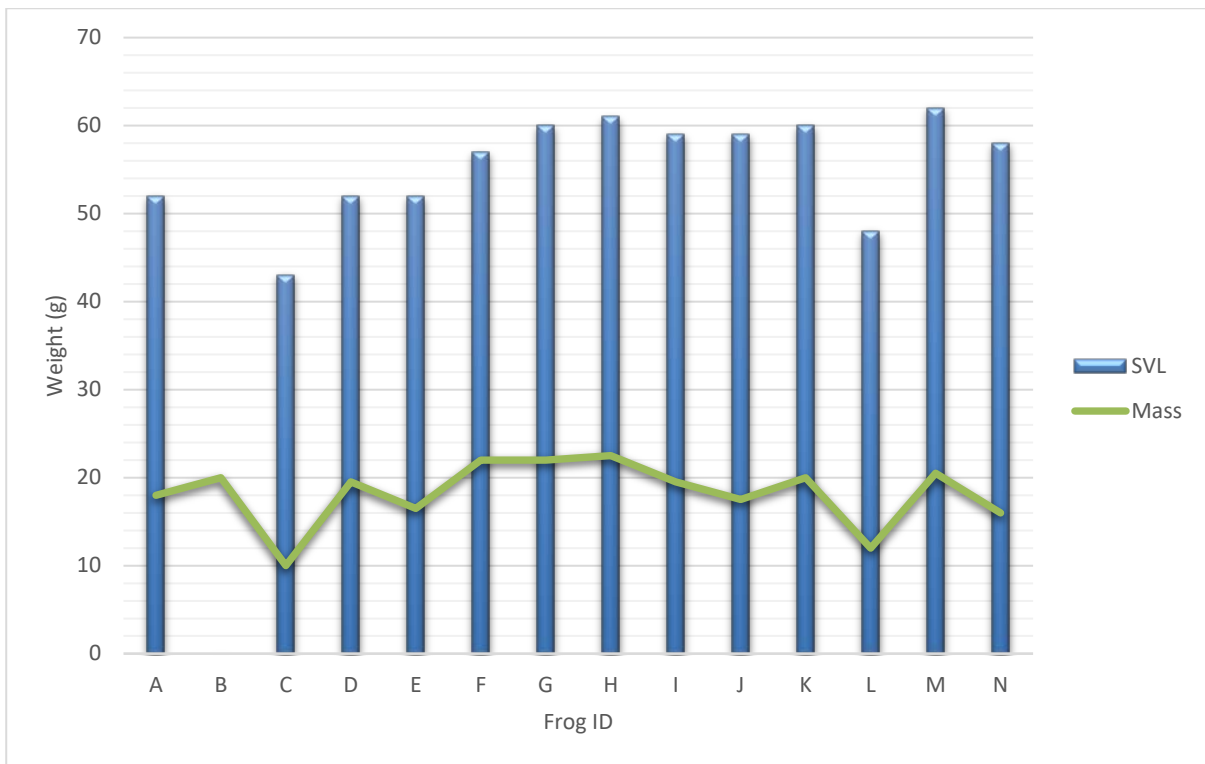


Figure 1. Snout-vent-length (SVL) and body mass of each frog individual (A-N), which was tagged.

The diversity of freshwater macroinvertebrates in the streams of Danum Valley, East Malaysia

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Abstract

A large proportion of the world's freshwater is found within the tropics. However, our current understanding of the diversity of freshwater macroinvertebrates in Malaysia is incomplete, with many species unknown to science. This study focused on how the family diversity and abundance of freshwater macroinvertebrates varied with substrate type and rate of water flow in three streams in Danum Valley Conservation Area, East Malaysia. There was minimal variation in the number of families as flow rate and substrate type altered. However, the families present varied, particularly between substrates, with most families inhabiting one or two substrate types. This is likely due to the energetic cost associated with evolving the necessary locomotive, feeding and breathing apparatus for the varied substrate types. Variation in families present with flow rate was much less distinct, likely due to the limited difference in the flow speeds across the streams.

INTRODUCTION

Only 0.3% of the world's water is available as freshwater on the Earth's surface; but these systems support ~10% of the Earth's fauna (López-López & Diaz, 2015; National Geographic). Aquatic macroinvertebrates are widespread with high abundance across the world's streams, with communities varying with local conditions. Within streams insects make up ~90% of the benthic organisms, with ~4% of insect species spending part or all of their lifecycle in streams (Texas Commission on environmental quality). The remaining 10% are crayfish, clam, snail, worm and leech species.

Aquatic macroinvertebrates occupy a range of habitats in freshwater systems. This has led to the development of a range of adaptations for locomotion, feeding and breathing to enable the colonisation of all habitat types found within stream systems. Different environmental adaptations acquired by aquatic macroinvertebrates include: skating (surface-dwellers), planktonic living, diving (for food), swimming, clinging to objects, climbers (on plants) and burrowers (Merritt & Cummins, 1995). Aquatic invertebrates also display a range of feeding techniques, including: predation, piercing (suck fluids out of plants), scraping (remove material from hard surfaces), filtering, gathering (of fine particulates) and shredding (of plants, fungi, bacteria and algae). The feeding technique adopted by a species is closely related to the environment they are found in.

The analysis of the community structure and feeding mechanisms of species present in an area can provide an indication of the resources present and environmental disturbances in an area. This is because differing

macroinvertebrate species have varying tolerances to chemical, physical and biological agents. Therefore, the species present/absent indicate the condition of a stream. Macroinvertebrates can also be used to represent the ecological community in a system due to their importance in the transfer of energy from primary producers to higher trophic levels, due to their high diversity and abundance (López-López & Diaz, 2015).

A large proportion of the world's freshwater systems are found in tropical regions, and in Malaysia they are particularly diverse. However, in the Malaysian region many species of freshwater macroinvertebrates have yet to be described and there are likely phyla which have yet to be discovered (Yule & Yong, 2004). There are groups within the freshwater invertebrates that have been well studied in Malaysia, such as protozoa and mosquitoes, as they are vectors of human diseases. It is the organisms with minimal known impact on humans where research has been lacking. Recently, there has been an increase in the number of publications describing the species present for individual families or orders in localised areas, but these are generally larger, more 'charismatic' species, such as crabs and snails. Much research is required to understand the 'true' diversity of Malaysian freshwater systems, the ecology of species and their behaviour.

This study focused on the diversity of aquatic macroinvertebrates in three streams within the Danum Valley Conservation Area (DVCA). The aim of the study was to gain an understanding of how the diversity and abundance of the freshwater macroinvertebrate families are related to the physical attributes of freshwater systems, in particular, rate of water flow and substrate type.

METHODS

Study area

This study was carried out in primary tropical rainforest in DVCA, Sabah, Malaysia. Data were collected at three streams: W6S5 (N4°57'40.21" E 117°47'41.06"), Kalison (N 4°58'16.81" E117°48'58.72") and Palum Tambun (N4°57'41.94", E117°48'24.59") between the 18th and 22nd of October 2018 (Figure 1).

Data collection

Data were collected at 20 metre intervals along a 200 metre transect. Side pools were excluded from the study. At each sampling point the abiotic factors: pH, temperature, turbidity and percentage of dissolved oxygen in the water were measured. The biotic variable, percentage canopy cover was also measured. The stream characteristics: depth, width, substrate and rate of water flow were recorded. The substrate at each sampling point was recorded as either: sand, gravel, pebbles (<5 cm), small rocks (<10 cm), medium rocks (<20 cm), large rocks (>20 cm) or boulders. Kick-net sampling was used to collect aquatic macroinvertebrates (Figure 2). Methodologies produced by the Freshwater Biological Association (unknown) and Frost *et al.* (unknown) were used for kick-net sampling. The collected material was poured

into a tray and two people separated the macroinvertebrates from any debris for ten minutes or until no further invertebrates could be found (whichever was first). The aquatic macroinvertebrates were identified to the taxonomic level Family, using an optical microscope.

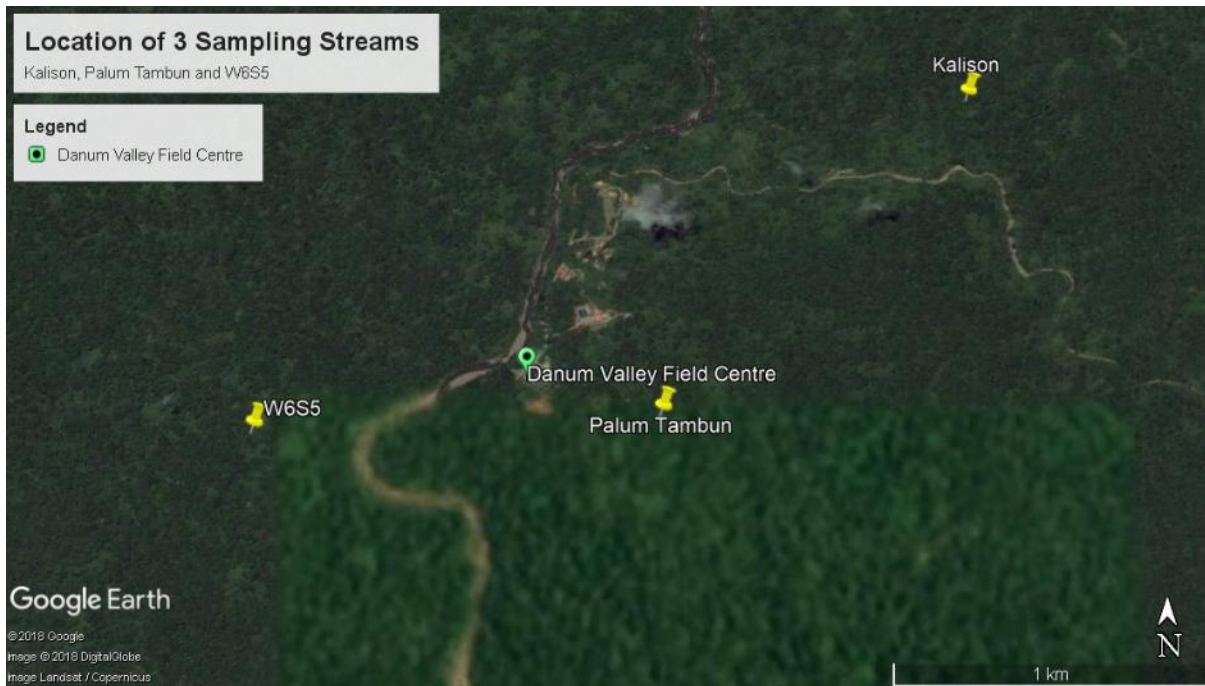


Figure 1. Location of W6S5, Kalison and Palum Tambun streams in Danum Valley Conservation Area, Sabah, Malaysia (Google Earth, 2018).



Figure 2. Collection of aquatic macroinvertebrates using kick-net sampling in the streams, Kalison (left) and W6S5 (right) in Danum Valley, Sabah, Malaysia

Statistical analysis

Data analysis were carried in R (R Core Team, 2018). To assess whether the three sampling streams could be analysed together, ANOVA was used to analyse the variation in pH and temperature between the streams. A TukeyHSD test was carried out to determine between which streams the temperature varied. To analyse the variation in family diversity with flow rate and substrate type an ANOVA was carried out.

To investigate the relationship between diversity of families with water depth and dissolved oxygen, a scatter plot was produced. For all statistical analyses the suitability of the model was assessed. A log transformation was carried out to improve the fit of the data to the model when assessing the temperature variation between streams.

RESULTS

There were 33 sampling points split evenly between the three streams, with 301 specimens collected, spanning 49 families. The average number of specimens collected per family were six. The maximum number of specimens collected for one family was 48; the family being Heptageniidae. The poorest represented families consisted of single specimens, for which there were 17 families.

There were no major difference in the pH between the three streams (ANOVA $F_{(2,28)} = 0.018$, $p = 0.982$). The average pH for the three streams had a range from 7.53 to 7.55. The temperature difference between W6S5, and Kalison and Palum Tambun was noteworthy (ANOVA $F_{(2,30)} = 0.018$, $p = 3.12e^{-05}$, Table 1). The stream at W6S5 had a higher water temperature than Palum Tambun and Kalison. The temperature of the water between Kalison and Palum Tambun did not massively vary. The average temperature at Kalison was 24.61 °C and 24.04 °C at Palum Tambun.

Table 1. TukeyHSD analysis showing the significance of the differences between the water temperature of three streams in Danum Valley, East Malaysia

Streams being compared	P value
P_Tambun and Kalison	0.0871530
W6S5 and Kalison	0.0080827
W6S5 and P_Tambun	0.0000198

The average number of families per sample site was highest for sites with a fast rate of water flow and lowest for sites with an intermediate rate of flow (Figure 3). However, the difference in family diversity with flow rate was not significant (ANOVA $F_{(2,30)} = 0.975$, $p = 0.389$). There were fifteen families with specimens only from fast-flowing water; two families with specimens from intermediate-flowing water only and eight

families with specimens from slow flowing water only (Figure 4). However, there were ten families with specimens from all flow types and nine families with specimens from two of the flow-types.

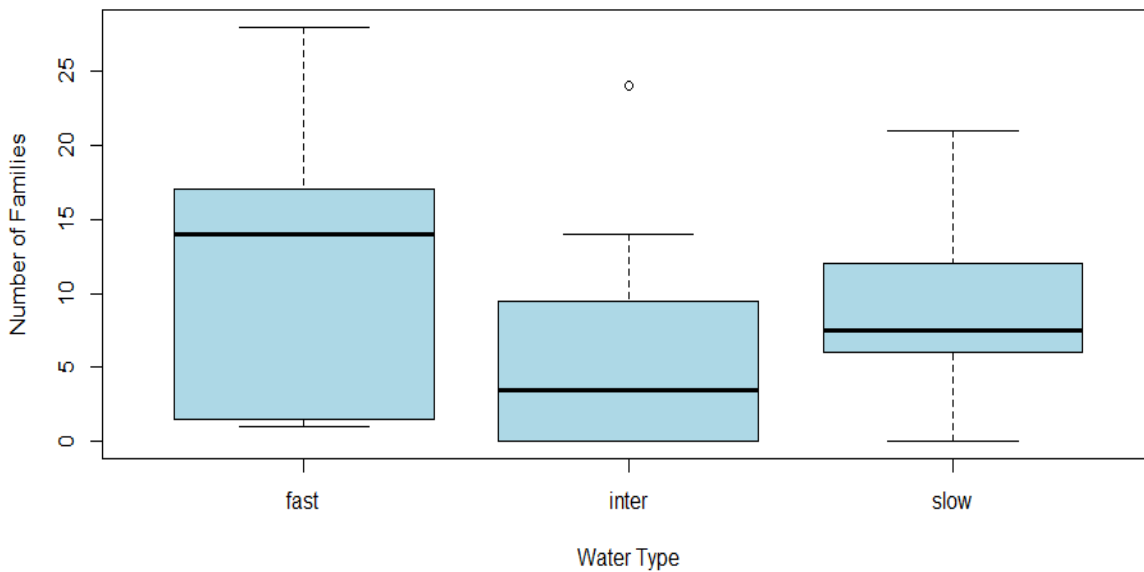


Figure 3. The diversity of freshwater macroinvertebrate families in three categories of flow rate: fast, intermediate and slow in streams at Danum Valley Conservation Area, East Malaysia.

The diversity of families varied with substrate. Sites where the main substrate was medium sized rocks had the highest average family diversity. The lowest average family diversity was when the main substrate at the sampling site was a boulder (Figure 5). The family found in the greatest range of substrates was Pachychilidae (six substrates). More than 70% of families were found in only one or two of the substrate types (Figure 6).

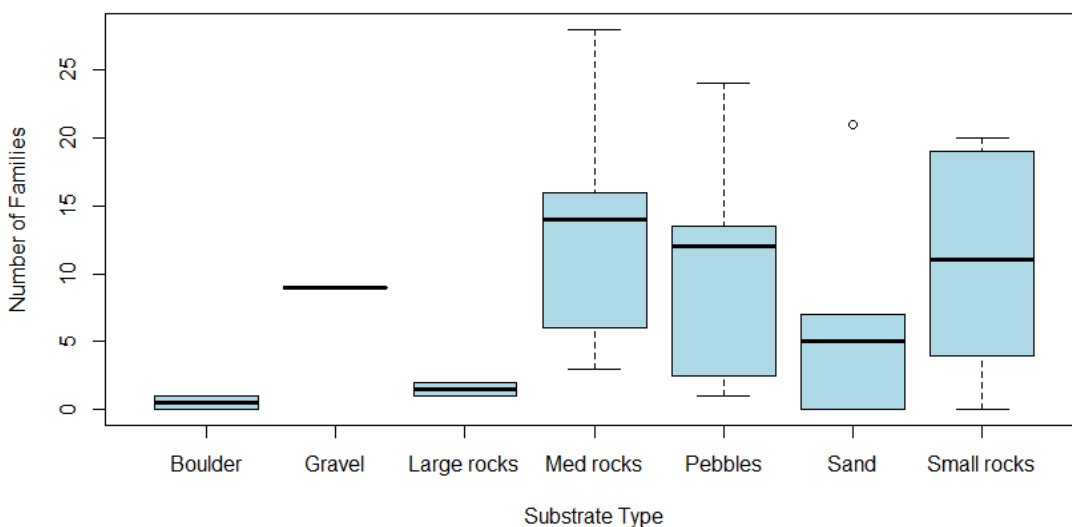


Figure 5. The diversity of freshwater macroinvertebrate families in seven categories of substrate in streams at Danum Valley Conservation Area, East Malaysia.

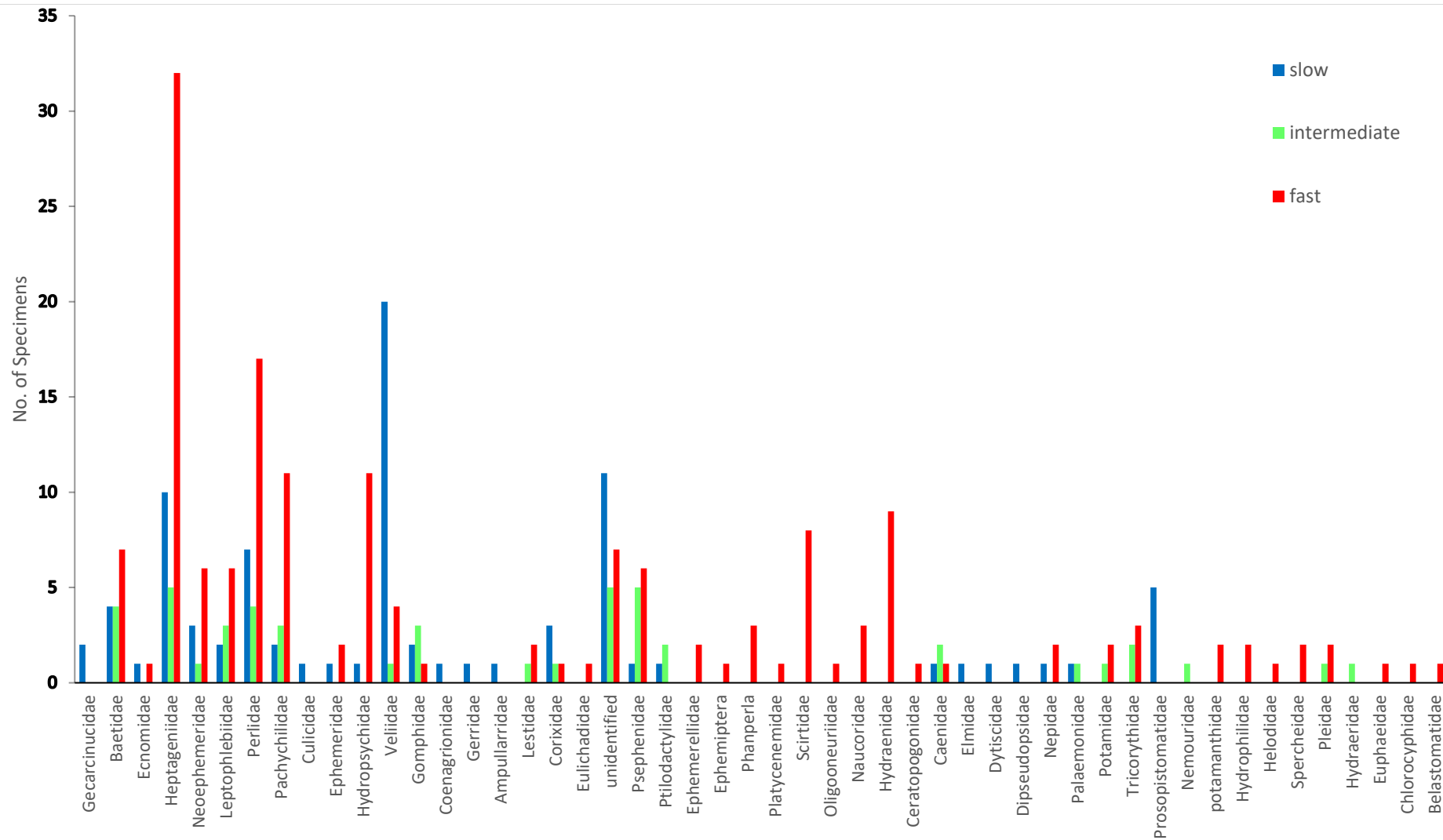


Figure 4. The distribution of specimens within families of freshwater macroinvertebrates with flow rate for streams in Danum Valley, East Malaysia

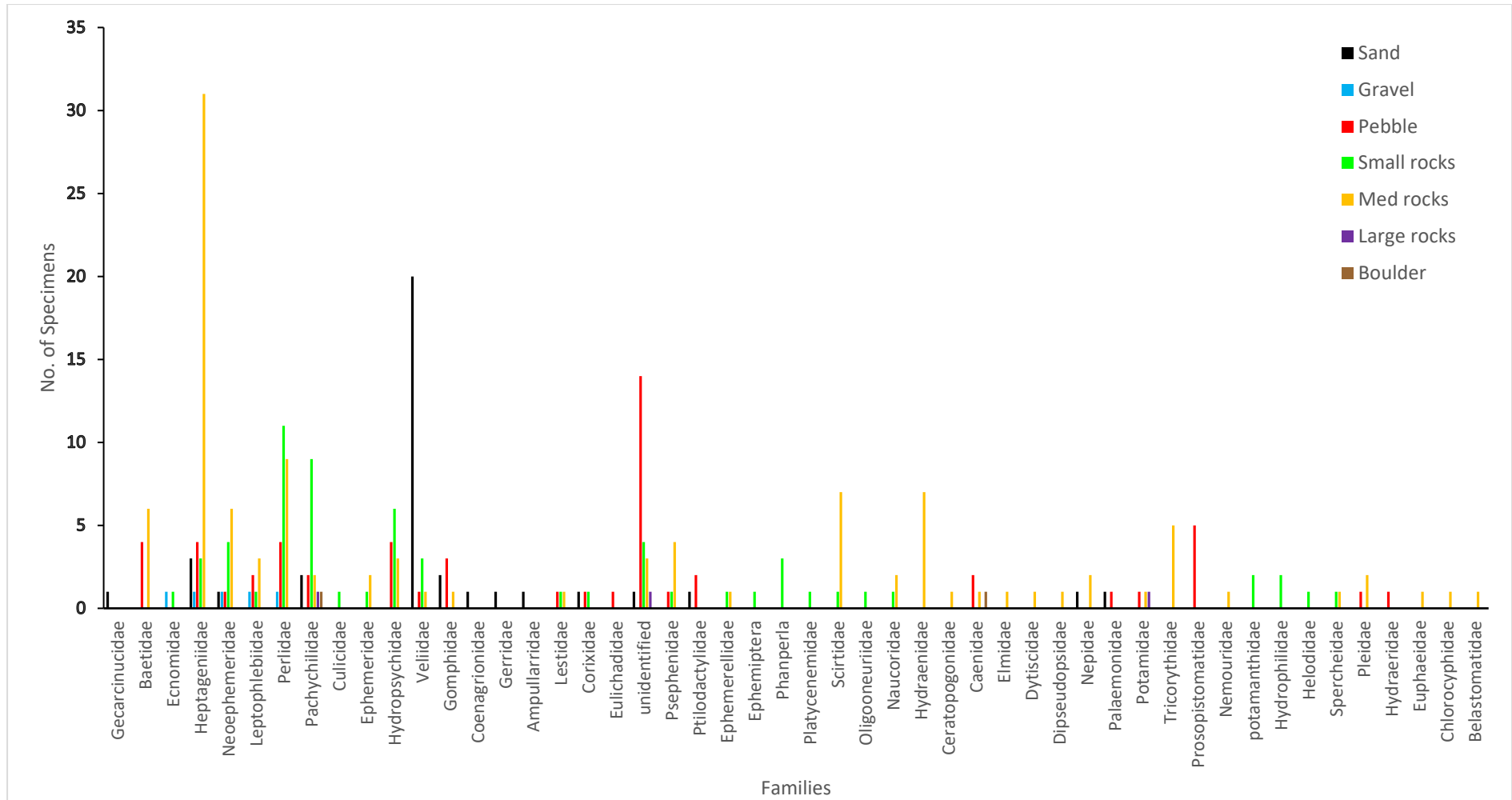


Figure 6. The distribution of specimens within families of freshwater macroinvertebrates with seven categories of substrate for streams in Danum Valley, East Malaysia

DISCUSSION

The data points collected for each stream were combined for analysis of how the diversity and abundance of families varied with stream characteristics. This was justified as all three streams were close together within an area of pristine primary forest within the DVCA, so are unlikely to be affected by human pollution and have the same underlying geology. There was minimal variation in pH (range of 0.02 between the averages) indicating that with respect to $[H^+]$ they were effectively the same. There was variation in the average temperature of the streams. However, this was likely largely due to the varied weather conditions during the study period, rather than a major underlying difference in the water catchments of the streams. The streams Kalison and Palum Tambun were both surveyed after storm events, and the influx of rainwater into the streams would be expected to lower their temperatures and this was found to be the case.

There was no major difference in the number of families found in the three flow rates investigated but there was variation in the families present in each flow type. The presence of a similar number of families across the flow rates indicates that there are no preferences for the inhabitation of a particular flow type, when considering freshwater macroinvertebrates as a whole. This may indicate that there are sufficient resources to support a similar number of families in all the flow types and no major difference in predation or starvation risk between the flow rates. There are families that appear to be 'generalists' in respect to flow rate. These families may be ones which are more species rich, resulting in a greater diversity of traits arising, enabling colonisation of a greater range of flow regimes. In general, the families with the highest number of specimens are 'generalist families'. Therefore, there is the potential that with further sampling, the 'specialists' would occupy a greater range of flow types. This seems particularly feasible when it is considering that kick-net sampling results in the collection of only ~20% of the organisms present (Frost *et al.*, unknown).

The methodology of substrate classification may have resulted in not all the substrates being equally well represented in the study. The substrate types medium rock, pebbles, sand and small rock were well sampled. Within these categories the substrate medium rock had the greatest average diversity and sand the lowest. The lower number of families in the substrate sand may be due to there being fewer food resources available, due to the lack of hard surfaces for primary producers. Also, the lack of a hard substrate results in fewer attachment sites for macroinvertebrates during flood events, causing the loss of organisms. The study found the majority of families were only on either one or two substrate types. This is potentially because it is not beneficial for a family to expend the energy/time to develop the variability in traits required for locomotion, feeding, breathing and predator avoidance in the different substrates. There were a few exceptions, such as the family Pachychilidae (gastropoda). They were found in six different substrates, including sand. This is likely a product of the method for the categorisation of the main substrate, as snails are not going to be able to attach to sand and were likely attached to a hard substrate within an area of predominantly sand.

This study revealed no clear relationship between the dissolved oxygen percentage and the number of families. This is potentially due to the high amount of dissolved oxygen across all sample sites; resulting in oxygen levels not having an important role in determining the presence of species in Danum. This is not surprising the location of the streams within an area of pristine primary rainforest.

The relationship between the number of families present and water depth was an upside down U-shape. The low family diversity at shallow depths may be due to an increased predation risk from terrestrially based predators and increased risk of becoming trapped in small pockets of water during low water levels. The peak family diversity between 20-30 cm could reflect a lower risk of predation, coupled with a continual supply of primary producers. The presence of a constant food source may be more restricted at greater depths in streams after storms due to increased turbidity, explaining the decline in family diversity.

This study has explored how diversity of mainly benthic macroinvertebrates varied with stream characteristics in the mornings and early afternoons. In a follow-up study it would be interesting to see how the families collected varied with time of day. Previous studies have indicated that during the day the probability of collecting certain species, such as crayfish is minimal. This is because they are in deep burrows during the day and return to the shallows in the evenings (Thorp & Rodgers, 2011). Side pools were excluded in this study as the kick-net sampling method was not suitable and the lack of time and resources to trial the Stove Piper Sample method suggested by Di Franco (2014). It would be interesting to collect data from side pools, as the ephemeral nature of these water bodies would require a different suite of adaptations to the species dwelling in streams.

CONCLUSION

This study found the streams of Danum Valley Conservation Area have a high diversity of families. When considering diversity at the family level, substrate type and flow rate did not affect the number of families present. This study likely captured only a small proportion of the true family diversity of the streams in Danum, due to the short period of field study. An in-depth future study of the pristine freshwater systems of Danum has the potential to generate species not previously known to science, especially considering the limited knowledge of freshwater aquatic diversity in the tropics.

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Species diversity and behaviour of Odonata in Danum Valley Conservation Area of Sabah, Malaysia

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Abstract

Odonata have always fascinated nature lovers and conservationists. They are huge and colourful, making their behaviour somewhat easy to observe. In addition, they have been extensively used as indicators for environmental quality in aquatic ecological units. The objectives were to evaluate the Odonata species diversity, species richness and behaviour, specifically *Camacinia gigantea*. Data collecting was carried out during 09:00 – 12:00 (noon) and 13:00 – 16:00 in two different ponds by scan for all species and focus observation for *Camacinia gigantea*. The light intensity and temperature were measured in every kind of the observation. The diversity of species of Odonata at each location was measured by Shannon Weiner index (H'). The relationship between the Odonata community and the environmental variables was done through canonical correspondence analysis. All analyses were conducted with the computer program PAST program 3.0. There were 23 species of Odonata of which Libellulidae and Coenagrionidae were the most dominant families. The diversity and richness of species of Odonata were higher in the shade pond than in open pond. The presence and abundance of some Odonata species were related to temperature and lux. Sitting and flying behaviours of all Odonata species, including *Camacinia gigantea*, could be observed from 09:00 to 15:30. Playing could be observed from 09:00 until 14:00, while mating and laying eggs could be observed from 09:00 to 10:45. Sitting behaviour was the most observed activity during the day time, alternating with a short time of flying to balance body temperature.

INTRODUCTION

Odonata can be recognised by their long, slender abdomen, large globular eyes which often make up a large portion of the head, short antennae, and long wings, which have a conspicuous node and usually a pterostigma (Neog & Rajkhowa, 2016). They are found in both temporary and permanent water bodies. They have strong association with water because of their aquatic larval stage. Adult males vigorously defend territories near water as these areas provide suitable habitats for the larvae to develop, and for females to lay their eggs. Their eggs develop in the water until going through the post larval stage. Adult Odonata spend their life on land. The larvae of dragonflies are considered as predators in freshwater ecosystems because they hunt the smaller insects, zooplanktons, other insect lava, and baby shrimp.

Odonata usually have a definite habitat preference, territorial behaviours and are sensitive to different environmental conditions such as temperature, oxygen levels, and amount of forest cover. Dragonflies are

huge and colourful, making their behaviour somewhat easy to observe. In addition, their larvae, adults and cast specimens have been used to assess the water environment conditions. They have been extensively used as indicators of environmental quality in aquatic ecological units.

Camacinia gigantea is the largest species of dragonfly in the family Libellulidae. In males, the hindwing is 44 to 48 mm in length and the total body length ranges from 53 to 56 mm. The male has a dark red body and the wing is dark red except for the distal one-third. The female is slightly larger and is yellow in colour. The basal wing colouring of females is pale and less extensive than that of the male (Figure 1). This species ranges from northern India through tropical Southeast Asia to New Guinea and it has been considered as a rare species in Singapore (Jaffar, 2012). However, this species can be found easily and considered as a common species in Danum Valley Conservation Area of Malaysia. The behaviour of this species has been observed in the pond in the Gaur exhibit of Singapore by Jaffar (2012). Since then, there was no comprehensive study on its behaviour. In the present study, we provide more data on the behaviour of this species.

The objectives of this study were to evaluate the Odonata species diversity, species richness and some behaviours in the two different ponds in Danum Valley Conservation Area. In addition, the behaviour of a common dragonfly species, *Camacinia gigantea*, was observed specifically.

Study area

This study was conducted during 18th - 22th October 2018 in the Danum Valley Conservation Area, Sabah, Malaysia. Two different habitats of dragonflies were chosen as our study sites based on different environmental conditions as follows:

The pond with open area (O) is located at 05°'89'115"N latitude, 05°49'111" E longitude and 181 m above the sea level. This pond has a total area of 11.10 square metres. It is located on the side road, west of the environmental education gallery, which is disturbed by humans sometimes. There are some trees growing around, with dense, tall trees growing on the west and some small trees growing at the northern and eastern sides of the pond. The western side of the pond is covered with grass without any trees. There are some floating plants covering about 20 percent of the water surface. There is no canopy cover (Figure 1).

The pond with shade area (S) is located at 05°'88'972"N latitude, 05°49'179" E longitude and 175 m above the sea level. This pond has a total area of 200 square metres. It is located about 200 m northeast of the previous pond. This pond is located in the forest and was not disturbed by any human activity. There are many species of plants of different sizes growing all around the pond. There are

some floating plants covering about 40 percent of the water surface. Canopy cover in this area is 51 percent (Figure 1o, 1s).



Figure 1. The sampling sites of Odonata in Danum Valley (O: the pond with open area, S: the pond with shade area).

MATERIALS AND METHODS

Data collection

Data collecting was carried out 09:00 – 12:00 midday and 13:00 – 16:00 in two different ponds by scan and focus observation. The environmental factors including light intensity and temperature were measured by using a three-way meter and iButton thermocline in every kind of observation. The scan observation was conducted to record the number of individuals and species, and also the behaviour and activity of individuals for each species i.e. sitting, flying, playing (flying with other individuals), mating, eating and laying eggs. The scan observations were conducted in every 20 minutes. The focus observation recorded the behaviour of *Camacinia gigantea* i.e. sitting, flying, playing, mating, eating and laying eggs every 5 second for 20 minutes. When the scan observation was done but no individuals of *Camacinia gigantea* was found, we continued our scan observation every 20 minutes.

Data analysis

The diversity of species of Odonata at each location and time i.e. open pond in the morning (OAM), open pond in the afternoon (OPM), shade pond in the morning (SAM), shade pond in the afternoon (SPM) was measured by the Shannon Weiner index (H'). Examination of the relationship between the Odonata community and the various environmental variables was done through canonical

correspondence analysis. All analyses were conducted with the computer program PAST program 3.0. Behaviour was analysed used Excel 2016.

RESULTS

Species richness and species diversity of Odonata

In the present study, 5 families, 16 genera and 23 species of dragonflies were found, 11 species from the open pond and 17 species from the shaded pond. Family Libellulidae and Coenagrionidae were the two most dominant families, which contained 5 and 14 species respectively. Number of species in OAM and OPM were 10 and 9 species, while that in both SAM were 15 species.

Camacinia gigantea, *Neurothemis ramburii* and *Orthetrum testaceum* were the most common species that was found in both areas and times (OAM, OPM, SAM and SPM). *Orthetrum testaceum* and *Pseudagrion praetarmisum* could be found in both areas in the same or different times. Interestingly, we found that *Orthetrum chrysis*, *Orthetrum glaucum*, *Rhyothemis triangularis*, *Tramea transmarina*, *Agrionemisa femina*, *Pseudagrion microcephalum* could be found only in the open area, while *Anax* sp., *Agrionoptera insignis*, *Neurothemis terminate*, *Orthetrum pruinatum*, *Tetrathemis irregularis*, *Tyriobapta torrida*, *Zygomma obtusum*, *Lestes praemorsus*, *Coelicia nigrohamata*, *Copera vittata*, *Stenagrion dubium*, and an unidentified species could be found only in the shaded area. *Orthetrum testaceum* and *Agrionoptera insignis* were the most abundant species in the open and shade areas, respectively (Table 1).

The Shannon Weiner index indicated that the species richness of the dragonflies in OAM, OPM, SAM and SPM, were $H' = 1.94, 1.90, 2.49$ and 2.34 , respectively. We found that the species richness within the area in different times was not different. However, the species richness between two areas in the same and different times were significantly different (Table 1).

Table 1. Species richness and species diversity of Odonata in Danum Valley

No.	Taxa	Open (O)		Total of Individual	Shade (S)		Total of Individuals
		AM	PM		AM	PM	
	Anisoptera						
	Aeshnidae						
1	<i>Anax</i> sp.					1	1
	Libellulidae						
2	<i>Agrionoptera insignis</i>				33	32	65
3	<i>Camacinia gigantean</i>	21	11	32	17	9	26
4	<i>Neurothemis ramburii</i>	19	17	36	13	2	15
5	<i>Neurothemis terminate</i>				14		14
	Libellulidae						
6	<i>Orthetrum testaceum</i>		1	1	1		1
7	<i>Orthetrum chrysis</i>	2	6	8			
8	<i>Orthetrum glaucum</i>	3	10	13			
9	<i>Orthetrum pruinatum</i>				8	7	15
10	<i>Orthetrum testaceum</i>	18	35	53	36	13	49
11	<i>Rhyothemis triangularis</i>	21	15	36			

12	<i>Tetrathemis irregularis</i>				10	3	13
13	<i>Tramea transmarina</i>	7	4	11			
14	<i>Tyriobapta torrida</i>				19	4	23
15	<i>Zyxomma obtusum</i>					3	3
	Zygoptera						
	Lestidae						
16	<i>Lestes praemorsus</i>				17	9	26
	Platycnemididae						
17	<i>Coeliccia nigrohamata</i>				23	16	39
18	<i>Copera vittata</i>				2	1	3
	Coenagrionidae						
19	<i>Agriocnemis femina</i>	2		2			
20	<i>Pseudagrion microcephalum</i>	4	6	10			
21	<i>Ceriagrion chaoi</i>	2		2	9	7	16
22	<i>Stenagrion dubium</i>				15	20	35
23	Unidentified species				6	6	12
Total of individual		99	105	204	223	133	356
Total of species		10	9	11	15	15	17
Shannon Index (H)		1.94a	1.90a		2.49b	2.34b	

(Compare diversity α : 5%)

Correlation between environmental factors and species of Odonata

The environmental factors such as light intensity and temperature were correlated positively with the species of Odonata. The changing of temperature during day time had the most influence on the appearance of *Rhyothemis triangularis* and *Tramea transmarina*, while light intensity had the most influence on the appearance of *Orthetrum glaucum*, *Orthetrum chrysis* and *Pseudagrion microcephalum* in the open pond at the morning. Light intensity and temperature were not too influential to *Camacina gigantea* which could be found in both areas and times during the day (Figure 2).

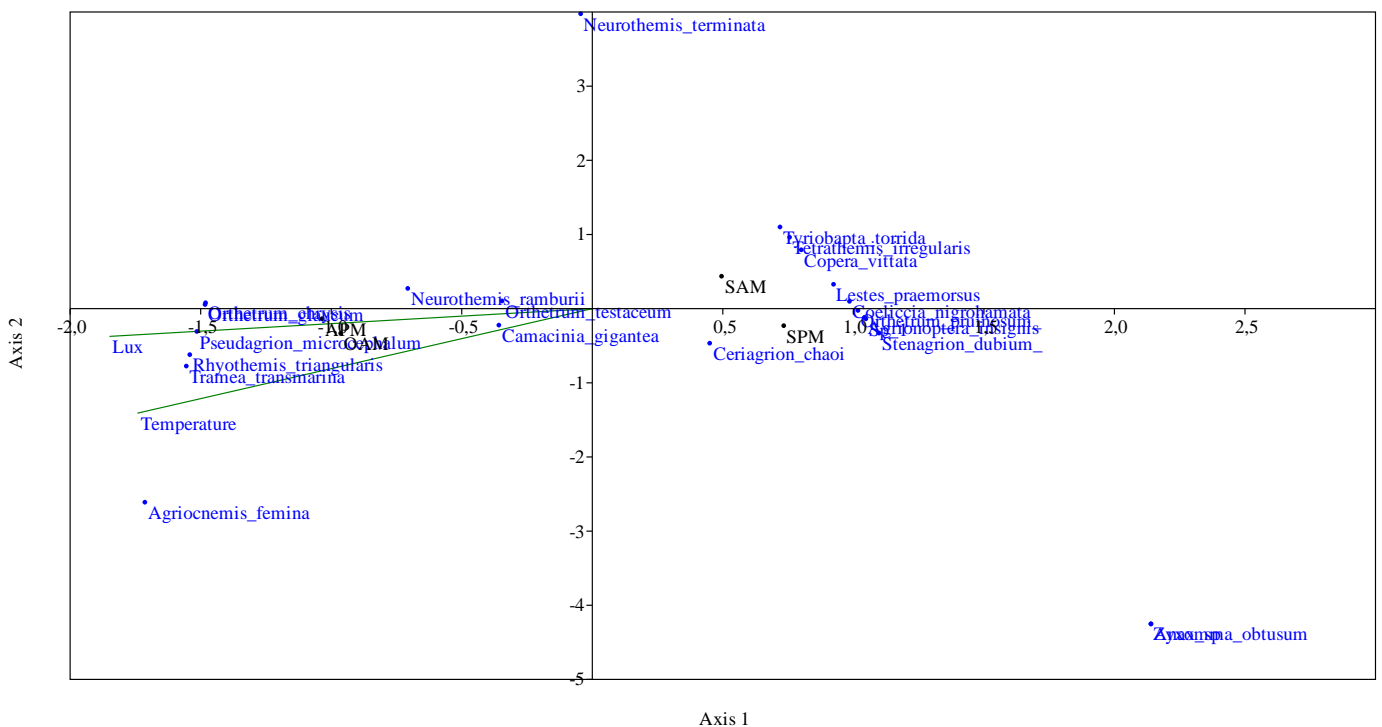


Figure 2. Ordination diagram correlation between light intensity, temperature and Odonata species.

Behaviour of Odonata species by scan observation

The Odonata spend the most time for sitting, flying and playing. There was only little time used for mating and laying eggs, and eating activity was not observed (Figure 3a). In detail, a lot of species in the suborder Anisoptera were flying, playing and sitting than those in the suborder Zygoptera. In contrast, those in the suborder Zygoptera spent most their time for mating (Figure 3b).

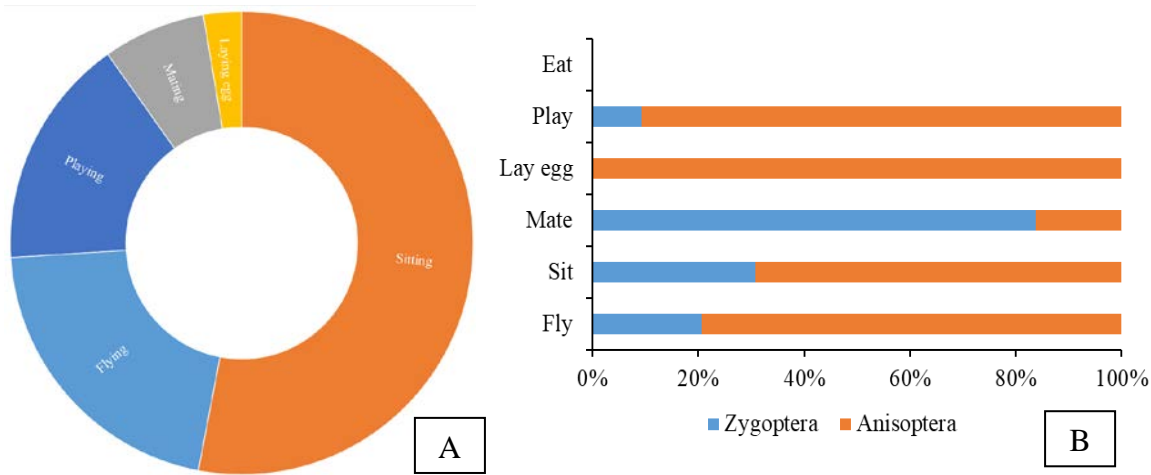


Figure 3. Proportion of behaviour activity of Odonata individuals. A. The behaviour of all Odonata individuals, B. The behaviour of Zygoptera and Anisoptera suborder.

Behaviour of *Camacinia gigantea*

Sitting comprised the largest proportion of activity of *Camacinia gigantea* along the day, although they did show flying activity. Sitting, flying, playing, mating, laying eggs and eating comprised 53, 21, 16, 7, 3, and 0 percent respectively in terms of time during 09:00 – 15:30 in all areas. Sitting and flying behaviours could be observed since 09:00 to 15:30. Playing could be observed from 09:00 until 14:00, while mating and laying eggs could be observed in the morning time from 09:00 to 10:45 (Figure 4).

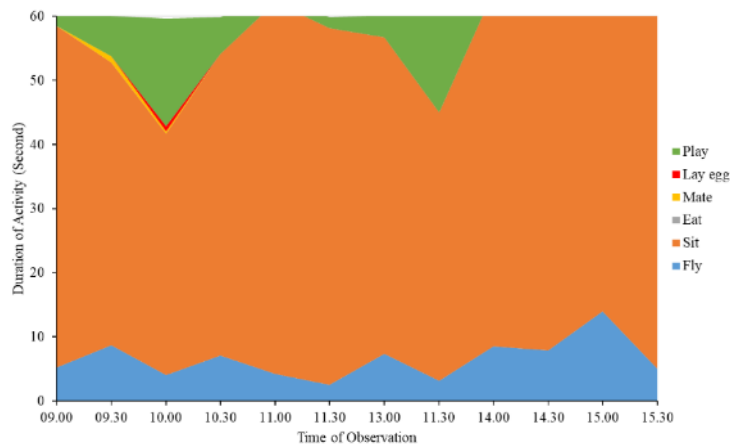


Figure 4. Behaviour of *Camacinia gigantea* in two open pond and shade pond during day time.

Interestingly, on 22th October 2018 at 09:50, one male and female were observed at the shade pond for mating activity. Three male individuals were flying for 12 minutes above water surface of the pond. Thereafter, one female from the vegetation joined one male in a mating wheel for 1 minutes and 15 seconds. At 10:22 the other male and female were flying and joining each other for 16 minutes, and then they mated for 30 seconds. At 10:40 one female started laying eggs on the surface of the water, at the side of the pool with vegetation that extended into the water. The female individual dipped her anal appendage into the water and released her eggs for 45 seconds. After egg laying was finished at 10:41.

The temperature and lux affected the sitting activity period of *Camacinia gigantea*. The sitting period flow followed the temperature and the lux conditions. The other activity i.e. flying and playing were in contrast to sitting activity.

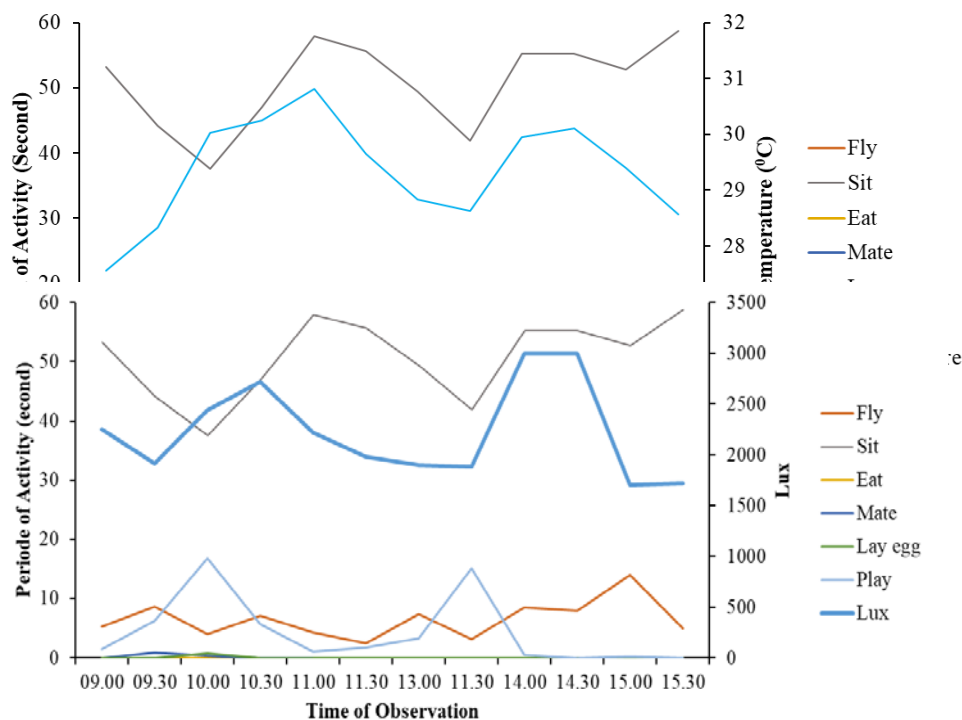


Figure 5. The correlation between behaviour activity and the environment factors.

DISCUSSION

Based on this study, 23 species of the Odonata in the Danum Valley Conservation Area were found in the pond habitats. Our data contributes to the biodiversity database of this area. The number of species in the open and shaded areas was different with 13 and 17 species respectively, which is perhaps caused by the environmental differences between the two areas. Characteristics of the vegetation (submerged, floating, emergent, or waterside) are also important. Adult Odonata may require emergent or waterside plants to use as perches and others may need specific submerged or floating plants on which to lay eggs. In the open area,

floating plants only covered 20 percent of the water surface while those in the shade areas covered 40 percent, which may provide more habitats for feeding, hunting, escaping the enemy for many species.

Libellulidae and Coenagrionidae were the dominant families with 14 and 5 species respectively. These two families mostly live in the lentic water covered by floating plants, whereas Aeshnidae and *Anax* sp., which might be rare, mostly live under fallen leaves and their larva hide under the mud or sand in the pond. We expect to see more individuals of these species when sediments in the pond are collected.

Excluding mating behaviour, the Odonata spent most of their time during the day resting on the branches of trees, and sometimes flying or basking in the sun in their habitats. The light intensity and temperature were influential factors on the distribution and behaviour of different species of dragonflies. The flight muscles need to be kept at a suitable temperature for dragonflies to be able to fly. Being cold-blooded, they can raise their temperature by basking in the sun. Early in the morning, they may choose to perch in a vertical position with the wings outstretched, whereas in the middle of the day, a horizontal stance may be chosen. Another method of warming up used by some larger dragonflies is wing-whirring, a rapid vibration of the wings that causes heat to be generated in the flight muscles (Berger, 2004).

Our study on the behaviour of *Camacinia gigantea* agrees well with that described by Jaffar (2012) which indicates that this dragonfly mostly rests during day time. Jaffar (2012) also observed the mating and ovipositing behaviour of this species and found that before mating, the male and female join each other (to fly and play), and after a few seconds, both male and female fly separately, after which the female lays eggs. We considered that the joining together of the male and female might be a “*courtship behaviour*” which may happen just before mating. Different individuals and species of Odonata may have different mating behaviour. More observation and details on the mating behaviour of this species is needed.

On the other side, this research indicates that habitat change will affect the diversity of Odonata species. Some species of Odonata cannot be found in disturbed habitats. Changes in temperature and lux may affect some species such as *Rhyothemis triangularis*, *Tramea transmarina*, *Orthetrum glaucum*, *Orthetrum chrysis* and *Pseudagrion microcephalum*.

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We are sincerely grateful for Dr Kevin Wallace for guiding us to develop our ideas. We would like to thank Mr Dede Deddey for helping us in selecting the sampling areas. We thank the Tropical Biology Association for giving us a valuable opportunity to learn the new world of nature conservation in Borneo.

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APPENDIX

Common Odonata species in open pond and shade pond



Camacinia gigantea (Libellulidae)



Neurothemis ramburii (Libellulidae)



Orthetrum testaceum (Libellulidae)



Orthetrum pruinosum (Libellulidae)



Rhyothemis triangularis (Libellulidae)



Agrionoptera insignis (Libellulidae)

Species diversity of butterfly in open and forested area in Danum Valley Conservation Area, Sabah, Malaysia

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Abstract

Butterflies are a taxon that is highly sensitive to habitat disturbance and most of them are threatened by forest modification. Understanding the species diversity of butterflies is crucial for monitoring the status of butterflies in an area. Passive sampling using baited traps and active sampling using butterfly nets was done in forested and open areas of Danum Valley Conservation Area (DVCA). The open area was found to have a higher species diversity and abundance compared to forested areas. There were 35 species found in total, seven of them are shared in both open and forested areas, six were only found in the forest whereas the remaining 22 species were found only in the open area. Butterflies prefer areas with more sunlight and a higher temperature mainly to regulate their body temperature and generate energy. It is evident that certain species were habitat specific and can only be found in the forested area but not in the open area and vice versa. It is important to identify the species and abundance of butterflies in DVCA for future management and habitat protection purposes.

INTRODUCTION

The hot and humid atmosphere of tropical rainforests hosts a diverse ecosystem with many endemic species ranging from large mammals to insects. The abundance of food plants and sunlight is favourable for many species of butterflies. There are 944 species of 272 genera among 5 families that have been identified in Borneo (Otsuka, 2001). Of them, 10% comprising of 90 species are endemic whereas 70% can only be found in the former part of Sundaland and 20% in Philippines, Sulawesi and the rest of the world. Butterflies roamed over a variety of habitats such as in forest areas and forest edges, by the streams and ridges as well as around the villages and farms.

Butterflies can be classified into three main groups, namely, lowland, low mountain and high mountain butterflies according to their presence on different level of elevation. Butterflies are a taxon that is highly sensitive to habitat disturbance and most of them are threatened by forest modification (Koh, 2007). The amount of light that entering the forest is vital in determining the life cycle of the insects. There is plenty of evidence showing that there is a higher number of butterflies in gap areas compared to the dense forest area (Hill, *et al.*, 2001).

This study aims to investigate the diversity of butterflies in Danum Valley Conservation Area. We assessed the following research questions: (1) Do open areas have a higher diversity of butterfly species

compared to forested areas? We predicted that open area, which has a higher canopy openness will be more favourable for butterfly species. (2) Do open areas have a higher abundance of butterflies compared to forested areas? We predicted that there will be a higher abundance of butterfly in the open area compared to the forested area. The results from this study would be crucial for monitoring the status of butterfly species in Danum Valley.

METHODS

Study area

This study was conducted in Danum Valley Conservation Area (DVCA), Sabah, Malaysia (Figure 1). DVCA is an informal protected area in the Ulu Segama Forest Reserve of primary, undisturbed lowland dipterocarp forest in Borneo. DVCA is an area equipped with an extensive trail system, permanent research plots, and other facilities such as a library and an educational centre.

Sampling design

The study was conducted over six days, from 18th to 23rd of October 2018. A line transect of 500 m length was set along the (1) forest and (2) open areas. The transect in the forested area was set along the forest trail whereas for the open area, it was set along the main road heading towards DVCA. Both passive and active sampling were used for this study. Passive sampling involves setting up baited traps along the 500 m transect. Each of the baited traps were set at 50 m apart from each other with a total of 10 baited traps in each transect. The bait used for all the traps was mashed banana and they were replenished daily. Active sampling was done along the same transect set for the passive sampling. There were five 10 m radius plot within the 500 m transect, each at 100 m apart and this required using butterfly nets to capture any butterflies found inside the plot. Both the passive and active sampling methods were carried out daily from 08:00 to 12:00 (noon).

Capture-mark-recapture method

All the butterflies that were captured in both the baited traps and active sampling were recorded and identified based on a field guide by Otsuka (2001). Each individual was given a signatory mark on their underside wings using a non-toxic animal marker (yellow for forested area and red for open area) for identification purpose. This allowed for the identification of recaptured individuals in order to see how far they travelled and how often they were recaptured.

Data recording

Microclimatic factors (i.e. temperature, relative humidity, light intensity and canopy openness) and time of capture for each individual were recorded for both sampling methods. The number of butterflies and moths

found in the bait or actively captured were recorded. Other disturbances to the baited traps for example, disturbed by macaques were also noted in the data sheet.

Data analysis

Species diversity was analysed by using the Paleontological Statistic software package (PAST) to calculate the Shannon's Diversity Index (H) at a 95% confidence interval (Hammer *et al.*, 2001). The number of species captured throughout the sampling period were calculated cumulatively and separated according to the two different areas they were captured in: forest and open.

The species diversity of butterflies in the forest and open areas were calculated using the Shannon's Diversity Index (H). This information was averaged over the sampling period to estimate a daily diversity index. A box plot was then plotted to present the results.

The species accumulation curve was plotted according to the cumulative number of species found as the day goes.

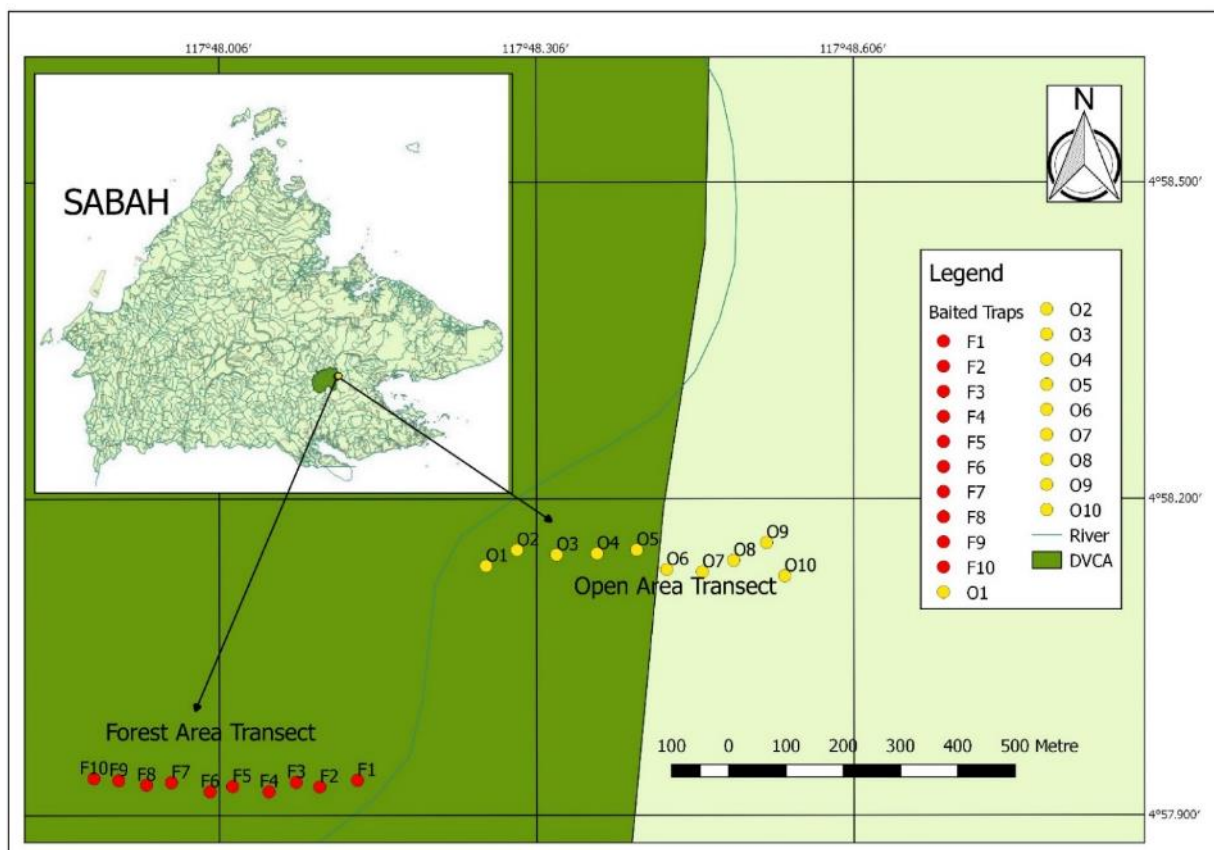


Figure 1. Map of the study area at Danum Valley Conservation Area, Sabah. The red dots and yellow dots represent the forested area and open area respectively.

RESULTS

Species richness and abundance

A total of 35 species from four families of butterfly were recorded (Table 1). Out of the 35 species, seven species were shared in both areas, six were only found in the forest whereas the remaining 22 species were found only in the open area. There were 124 individuals comprising of 55 and 69 individuals from the forested and open area respectively. The seven shared species found were: *Tanaecia munda*, *Discophora necho*, *Dophla evelina*, *Mycalesis horsfieldi*, *Mycalesis orseis*, *Ypthima baldus* and *Idea stollii*. Certain species such as *Ragadia makuta* could be found in high abundance, but only in the forested area. The same could be observed in open areas, for example, *Eurema lacteola* which was only found in open areas.

The results show that there is a higher number of species and abundance found in the open area compared to the forested areas. The species diversity estimate was significantly higher in the open area (95% CI; H = 3.02) compared to forest area (95% CI; H = 2.03).

Table 1. Species list of butterflies with the abundance in two study areas

Species	Family	Forested area	Open area
<i>Lexias sp</i>	Nymphalidae	1	0
<i>Appias lyncida</i>	Pieridae	0	1
<i>Bassarona dunya</i>	Nymphalidae	6	0
<i>Cepora iudith</i>	Pieridae	0	1
<i>Cethosia hypsea</i>	Nymphalidae	0	1
<i>Cirrochroa emalea</i>	Nymphalidae	0	2
<i>Coelites epiminthia</i>	Nymphalidae	0	1
<i>Discophora necho</i>	Nymphalidae	1	2
<i>Dophla evelina</i>	Nymphalidae	4	2
<i>Erites elegans</i>	Nymphalidae	1	0
<i>Euploea diocletianus</i>	Nymphalidae	0	1
<i>Euthalia iapis</i>	Nymphalidae	0	1
<i>Eurema lacteola</i>	Pieridae	0	8
<i>Eurema nicevillei</i>	Pieridae	0	10
<i>Eurema simulatrix</i>	Pieridae	0	2
<i>Idea stolli</i>	Nymphalidae	2	2
<i>Jamides celeno</i>	Lycanidae	0	2
<i>Leptosia nina</i>	Pieridae	0	1
<i>Moduza procris</i>	Nymphalidae	0	1
<i>Mycalesis anapita</i>	Nymphalidae	0	5
<i>Mycalesis horsfieldi</i>	Nymphalidae	4	6
<i>Mycalesis mineus</i>	Nymphalidae	0	1
<i>Mycalesis perseus</i>	Nymphalidae	10	0
<i>Mycalesis orseis</i>	Nymphalidae	1	3
<i>Nacaduba sp</i>	Lycanidae	0	1
<i>Neorina lowii</i>	Nymphalidae	2	0
<i>Yasoda sp.</i>	Lycanidae	0	1
<i>Papilio demolion</i>	Papilionidae	0	1
<i>Papilio nephelus</i>	Papilionidae	0	1
<i>Ragadia makuta</i>	Nymphalidae	20	0
<i>Rhinopalpa polynice</i>	Nymphalidae	0	2
<i>Tanaecia munda</i>	Nymphalidae	2	2
<i>Tanaecia sp.</i>	Nymphalidae	0	1
<i>Ypthima baldus</i>	Nymphalidae	1	6
<i>Ypthima pandocus</i>	Nymphalidae	0	1
Total Individual		55	69
Total Species		13	29
Shannon's Diversity Index (H)		2.03a	3.02b

The mean \pm SD species diversity of butterflies per day was higher in the open area (1.84 ± 0.966 , $n = 6$) compared to the forested areas (1.23 ± 0.506 , $n = 6$) (Figure 2). As the sampling days increase, there was also an increasing number of species found in our study sites (Figure 3).

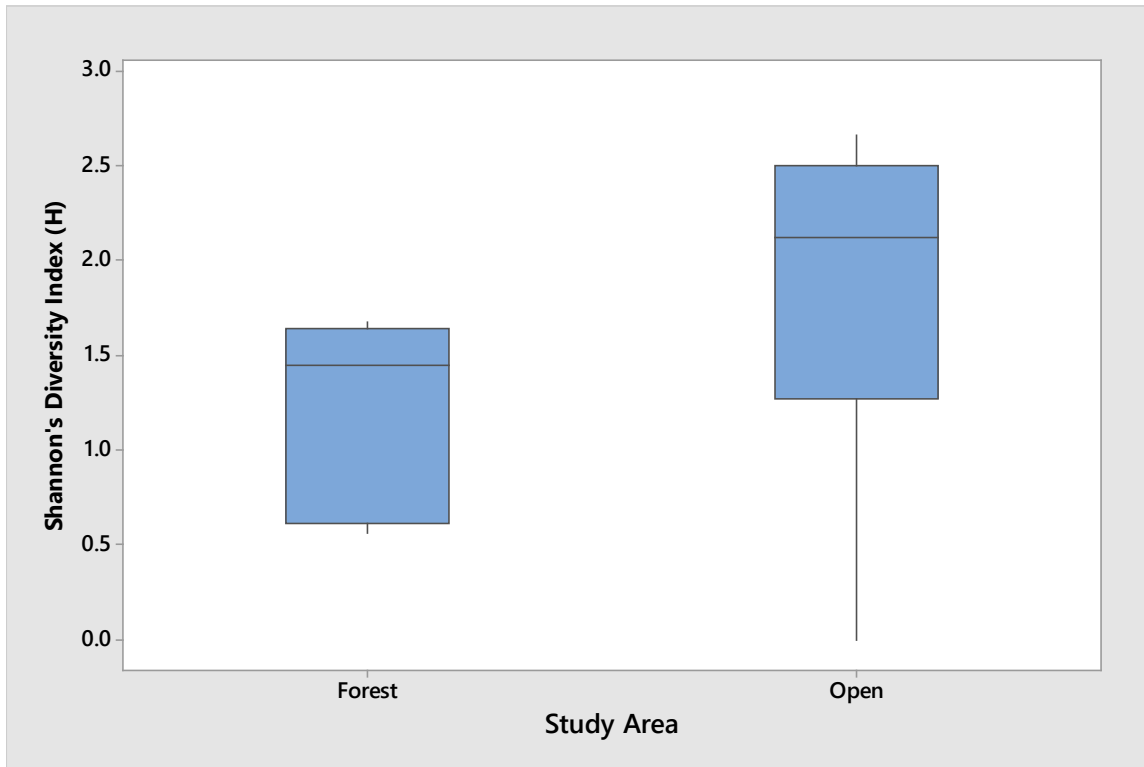


Figure 2. Boxplot diagram on the Shannon's diversity index of butterflies seen per day ($n = 6$) found in the forested and open areas of Danum Valley Conservation Area.

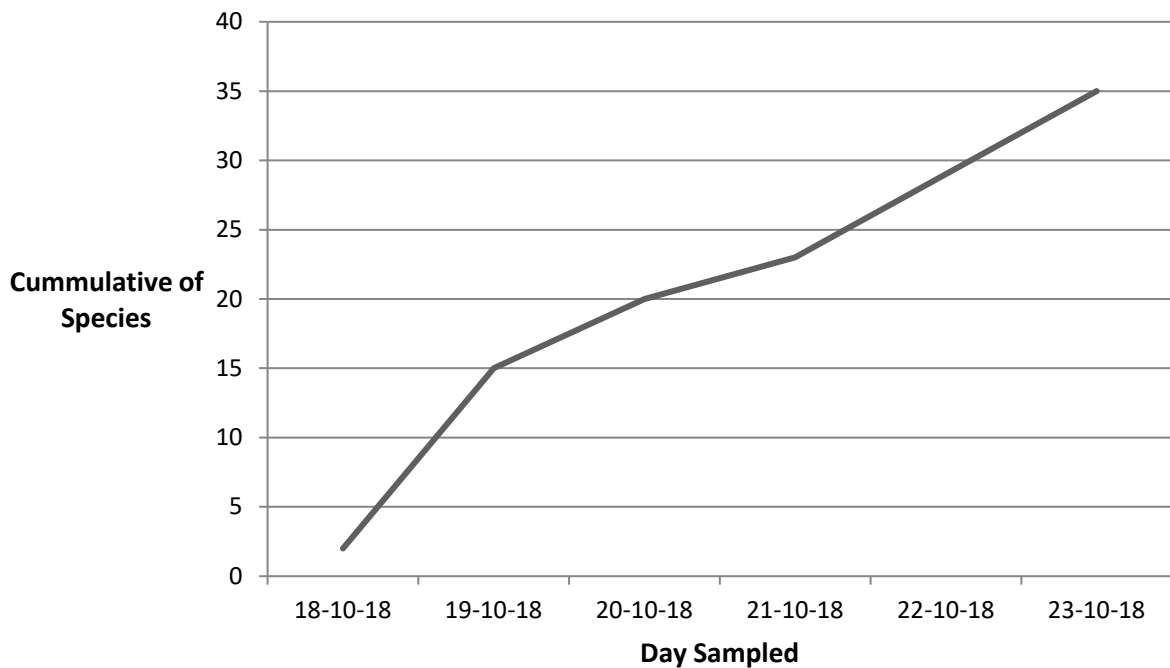


Figure 3. Species cumulative curve for butterfly species found across the study sites in six sampling days.

DISCUSSION

Butterflies in DVCA are more diverse and abundant in the open area compared to the forested area. This is mainly due to the higher sunlight availability in the open area (Houlihan *et al.*, 2013). Butterflies need sunlight to regulate their body temperature and generate energy. A study by Hill *et al.* (2001) shows similar results where more butterfly species are found in forest gaps and less in closed-canopy forests. Their study also supports our finding that certain species especially those in the forested areas are more habitat specific and could only be found in the closed-canopy forests. This shows the importance of the varied habitats for the different butterfly species.

Although the box plot in Figure 2 shows some overlap in species diversity between the forested and open areas, it could be explained by the absence of species caught in the open area during the first sampling day. The baited traps were set up only in the morning of the first sampling day and checked in the evening of the same day at 14:00. The weather started to become cloudy at that time and thus, many butterflies were inactive. This demonstrates that butterflies do favour the presence of sunlight and higher temperatures of the day. The butterflies found in the forested area of the first sampling day were all caught from the baited traps.

On many occasions, the open area baited traps were disturbed by macaques and that could have decreased the chances of getting many more species or individuals in the open area. The bananas used as baits were wiped clean and the traps were left open when checked in the morning.

According to the species cumulative curve, the number of species keeps increasing as the sampling days increase. This proves that there are many more species of butterflies that could be found in DVCA. Hence, more sampling sites or an increase in sampling effort should be considered in the future. For instance, having two transects in the open and forested areas each instead of one. However, this would also require more manpower and baited traps because the conducive time for conducting the sampling is in the morning up to noon. If there are three people in a team, only two transects can be surveyed each day. Future studies should also take into account the risk of disturbances from the macaques to the baited traps. Other than that, different types of baits could be used to study the differences in species towards the different type of baits.

Understanding the species diversity and abundance of butterflies in DVCA is important for the management and protection of their habitat.

ACKNOWLEDGEMENTS

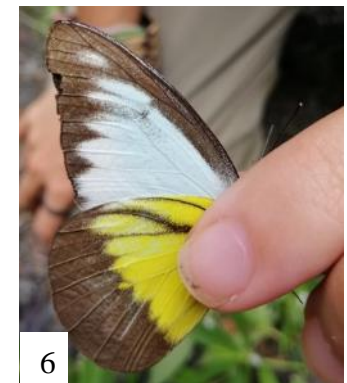
A special thanks to Tropical Biology Association and Southeast Asia Rainforest Research Partnership for giving us the opportunity to conduct this mini project in Danum Valley Conservation Area. We thank Dr Kevin Wallace (course coordinator), Dr Michael Brooke (lecturer), Dr Christopher Philipson (lecturer), and Dr Jennifer Sheridan (lecturer) for the countless guidance and constructive comments provided throughout

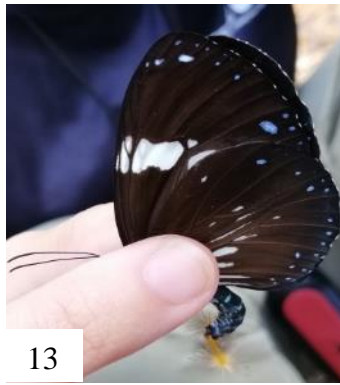
our project. We are grateful to Johnny Larenus (Project Manager of Danum Valley), research assistants Azzlin bin Sailim and Sisoan bin Mauhut for providing us the baited traps and guiding us throughout the whole project, especially in the field.

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APPENDIX









32



33



34



35

- | No. | Species |
|-----|-----------------------------|
| 1 | <i>Rhinopalpa polynice</i> |
| 2 | <i>Eurema simulatrix</i> |
| 3 | <i>Eurema nicevillei</i> |
| 4 | <i>Cirrochroa emalea</i> |
| 5 | <i>Cepora iudith</i> |
| 6 | <i>Appias lyncida</i> |
| 7 | <i>Eurema lacteola</i> |
| 8 | <i>Tanaecia julii</i> |
| 9 | <i>Leptosia nina</i> |
| 10 | <i>Coelites epiminthia</i> |
| 11 | <i>Euthalia iapis</i> |
| 12 | <i>Cethosia hypsea</i> |
| 13 | <i>Euploea diocletianus</i> |
| 14 | <i>Mycalesis anapita</i> |
| 15 | <i>Papilio nephelus</i> |
| 16 | <i>Mycalesis mineus</i> |
| 17 | <i>Nacaduba sp.</i> |
| 18 | <i>Ypthima pandocus</i> |

- | No. | Species |
|-----|-----------------------------|
| 19 | <i>Yasoda sp.</i> |
| 20 | <i>Jamides celeno</i> |
| 21 | <i>Moduza procris</i> |
| 22 | <i>Papilio demolion</i> |
| 23 | <i>Lexias sp.</i> |
| 24 | <i>Bassarona dunya</i> |
| 25 | <i>Neorina lowii</i> |
| 26 | <i>Mycalesis perseus</i> |
| 27 | <i>Ragadia makuta</i> |
| 28 | <i>Erites elegans</i> |
| 29 | <i>Ypthima baldus</i> |
| 30 | <i>Mycalesis orseis</i> |
| 31 | <i>Mycalesis horsfieldi</i> |
| 32 | <i>Dophla evelina</i> |
| 33 | <i>Tanaecia munda</i> |
| 34 | <i>Discophora necho</i> |
| 35 | <i>Idea stolli</i> |

1-22 open area
 23-28 forest species
 29-35 shared species

A comparison study of biomass changes due to drought event in primary and logged-over forest

Chigusa Renate Keller, University of Zurich, Switzerland

Gabriel Paul Hibberd, University of Bangor, United Kingdom

**Maliwan Namkhan, King Mongkut's University of Technology Thonburi,
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Project, Malaysia**

Abstract

Extreme weather events such as drought affect the ecosystem services of primary and logged-over forests. This study aims to assess recovery capability of primary and logged-over rainforests using above ground live tree biomass (AGB, t/ha) and its change related to drought and non-drought years. This study used three plots from Danum Valley Conservation Area (DVCA) and Innoprise-Face Foundation Rainforest Rehabilitation Project (INFRAPRO), respectively to represent primary forest and logged-over forest. In October 2018, trees were measured in plots of 0.1 ha size. AGB was modelled to compare the rate of change in AGB throughout different periods following a drought event that was identified between year 2015-2016. Primary forest plots have a higher total biomass than logged-over forest plots. However, the speed of biomass recovery is not significantly different between the two forest types. This implies that logged-over forests carry similar value regarding carbon storage as primary forest.

INTRODUCTION

In Malaysian Borneo, the primary land-use conversion in the last five decades has been for agricultural and housing developments. Efforts are made by the government to protect and safeguard remnants of primary forests from anthropogenic activities. Often, assumptions lean on forest value being decreased once they are logged. However, in recent years, logged-over forests have been recognised to be contributing towards diversity and ecosystem services as well. The value of logged-over forests for ecosystem services is a question of great concern for conservation and climate scientists alike.

The changing climate in tropical regions worldwide will affect both primary and logged-over forests. Wavering rainfall and extreme weather events dramatically change the availability of water and stability of the substrate in which trees grow. Extreme weather events, such as drought, can lead to an increase of tree falls and mortality. Detecting drought and assessing its effect on logged-over and primary forest is crucial,

especially in the context of global climate change. The ability of these forests to recover without permanent damage is essential for future regeneration management decisions.

Drought is a regularly occurring natural process observed in DVCA, a primary tropical lowland forest in South West of Sabah. Adjacent to DVCA is a mixed-dipterocarp rainforest that was severely logged-over in the 1980's and is undergoing rehabilitation. Therefore, the effect of this human disturbance can be studied in these forest types.

The objective of this study is to compare recovery capability of primary and logged-over rainforests using above ground live tree biomass (AGB) and its change during drought and non-drought years. Our findings can be a model for forests in similar climatic regions suffering from the same problems. This study addresses the following research questions: (1) Does higher biomass indicate higher resilience in terms of recovery growth from a drought event? We predicted that plots with higher initial biomass will experience less biomass loss due to drought event and plots with higher biomass will undergo more biomass regrowth after a drought event. (2) Do primary forests have higher resilience in terms of recovery growth from a drought event than logged-over forests? We predicted that plots in primary forest will experience less biomass loss due to the drought and will have more biomass re-growth after the event.

MATERIALS AND METHODS

Study area

In May 1995, DVCA was declared a Class 1 protected Forest Reserve by the Sabah Legislative Assembly. Later in the year 1999, DVCA was once more gazetted under The Cultural Heritage for Conservation Enactment 1998, as a Cultural Heritage Area. DVCA was established with aim to protect biodiversity, conducting research and education. INFAPRO (Innoprise-Face Foundation Rainforest Rehabilitation Project) is a 25,000 ha logged-over modified dipterocarp rainforest adjacent to DVCA. This forest was logged in the 1980's for timber production and been under recovery since the 1990's. This study used plots both within the DVCA and INFAPRO to represent primary forest and logged-over forest.

Data collection

Sampling design

There were a total of six study plots, three located in the primary forest, three in the logged-over forest (Figure 1): (i) Conservation Area 3 (04°57'771'N, 117°46'942'E), (ii) Conservation Area 4 (04°57'081'N, 117°46' 937'E), (iii) Conservation Area 5 (04°57'402'N, 117°47'681'E), (iv) Logged Forest 1981-1 (04°58'751'N, 117°52'458'E), (v) Logged Forest 1988-5 (04°59'055'N, 117°51'442'E), (vi) Logged Forest 1989-1 (04°58'710'N, 117°48'875'E). Each plot is circular and 0.1 ha in size. The data was collected in October 2018.

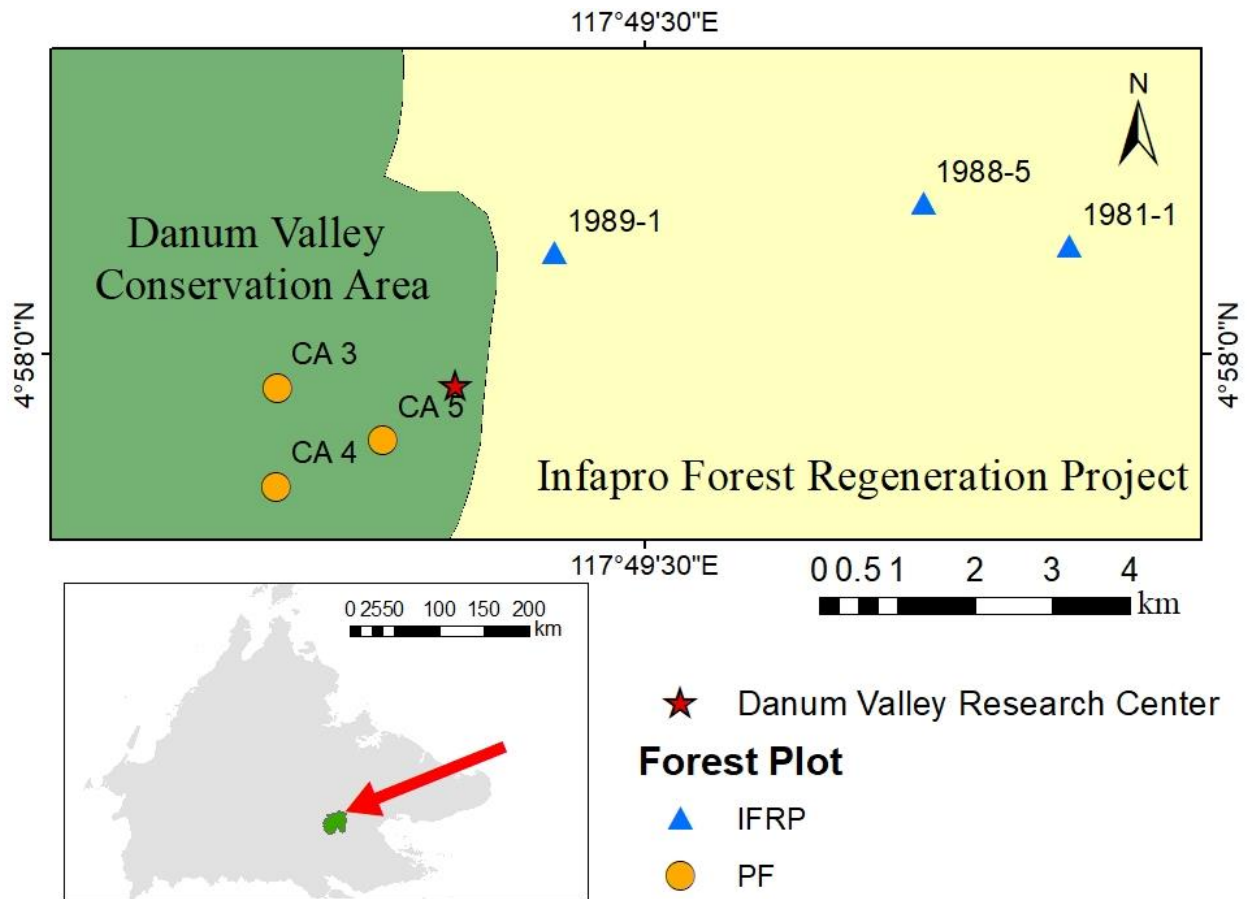


Figure 3. Location of forest plots (top) and location of DVCA in Sabah, Malaysia, Borneo (bottom left).

Tree measurements

Trees were measured from a circular forest plot area of 0.1 hectare (Figure 2). Trees with Diameter at Breast Height (DBH) > 20 cm were measured within the whole plot. Smaller trees with DBH > 2 cm and up to 20 cm were only measured within a subplot of radius 12.61 m (0.05 ha). In a smaller subplot with radius 2.00 m, every tree with DBH > 1 cm was measured. The individually labelled trees were located using azimuth and distance from the centre point of plot. The DBH of the trees were measured using a DBH tape. Calipers were used to measure live trees with DBH below 2 cm, then an average was obtained from two perpendicular measurements. Dead trees were not measured.

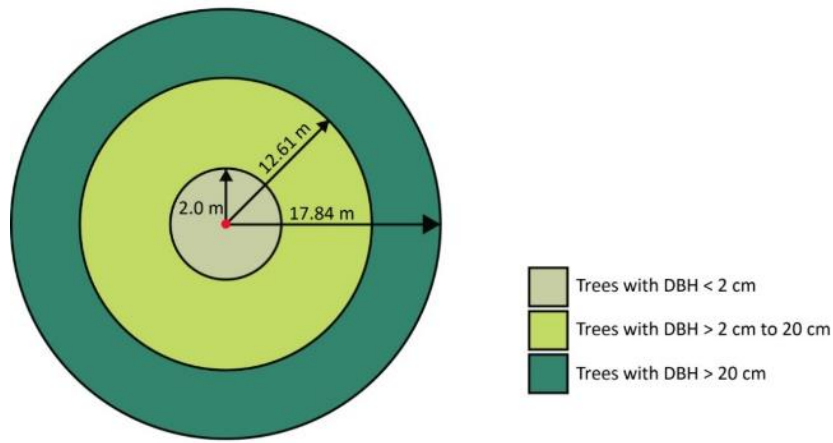
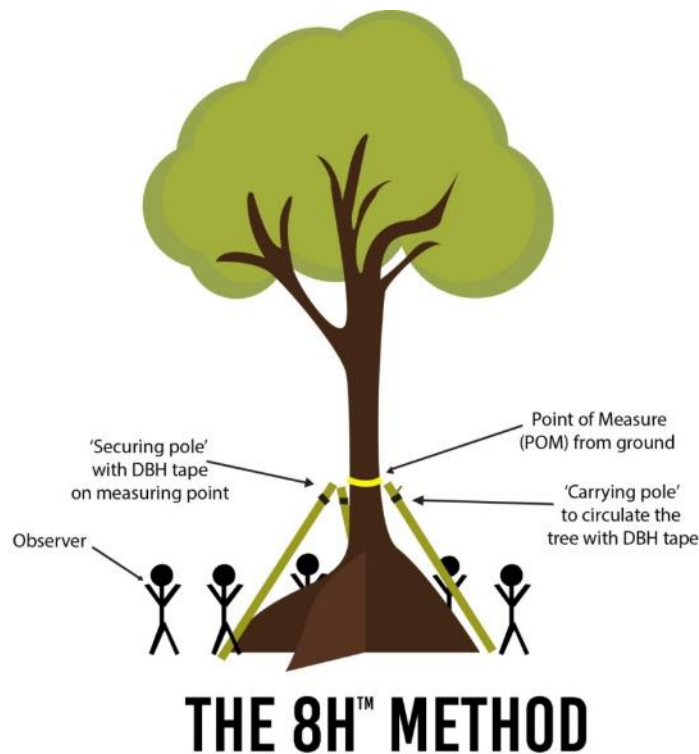


Figure 4. 0.1 ha forest plot and subplots with different DBH sizes.

In order to accurately and safely measure trees with tall buttress roots, the 8H™ Method was developed using 3 types of bamboo poles of 4 m length (Figure 3). A DBH tape is inserted in the slit on one end of the ‘securing pole’. The pole is then held up by one person to the measuring point on the tree trunk above the buttress roots. The ‘carrying pole’ has a small bamboo stick tied to its end, creating a small branch, from which the DBH tape is hanging down. A second person circles around the tree with the ‘carrying pole’ and crosses over the ‘securing pole’. A third person, equipped with the ‘lifting pole’, pushes up the DBH tape until it is horizontally wrapped around the tree, while the second person tightens the tape. Binoculars or a camera with enhanced zoom is required to read the measurements. The Point Of Measurement (POM) is then measured.



THE 8H™ METHOD

Figure 5. The 8H™ method for measuring the DBH of trees with tall buttress roots

Climate data

The climate data was obtained from the weather station at met hill at the Danum Valley Field Center. The data consisted of daily rainfall, temperature and relative humidity from 1st January 1985 to 31st December 2017.

ANALYSIS

Above ground live tree biomass

The above ground live tree biomasses (ABG) was calculated using Chave *et al.*, (2014) pantropical biomass equation:

$$AGB_{est} = 0.0673 * (pDBH^2H)^{0.976}$$

The wood density p was obtained from the Global Wood Density Database (Zanne *et al.*, 2009). The genus mean wood density was used for trees that had been identified. For trees with no identification, the mean wood density within the plot was used.

As tree heights were not measured, H was modelled using Christopher Philipson's (unpublished) height model estimation equation:

$$H = 89.53 * (1 - \exp(-0.0225 * DBH^{0.7383}))$$

For trees with point of measurement higher than 1.3 m, the tree tapering model by Cushman *et al.* (2004) and Jucker *et al.* (2017), was used to obtain the standardised DBH:

$$DBH_{1.3} = \frac{DBH_{POM}}{\exp(-0.029 * (POM - 1.3))}$$

Statistical analysis

One-way analysis of variance (ANOVA) was performed to compare the total above ground live tree biomass between primary forest and logged-over forest plots. Then, ANOVA was performed to compare the difference of biomass changes between primary forest plots and logged forest plots in two different periods of 2016 and 2018. Analyses were performed using R software.

RESULTS

Climate data

At DVCA, a reoccurring pattern of years with low rainfall can be observed in an interval of approximately 5 years (Figure 4). The years 2015, 2010, 2005 experienced low rainfall (approx. 2,250 mm per year). The years 1997 and 1985 showed more serious low rainfall events (< 2,000 mm and < 1,500 mm, respectively). The monthly rainfall data for the period of 2014 to 2017 shows that in 2015, there were several months with lower rainfall, as well as a five-month period of low rainfall from December 2015 to April 2016 (Figure 5).

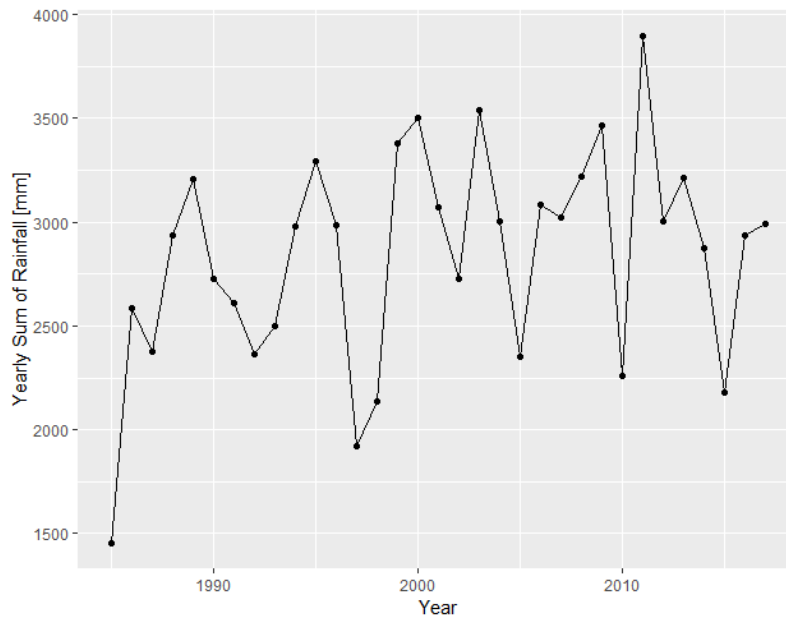


Figure 6. Annual rainfall data from 1985 until 2017.

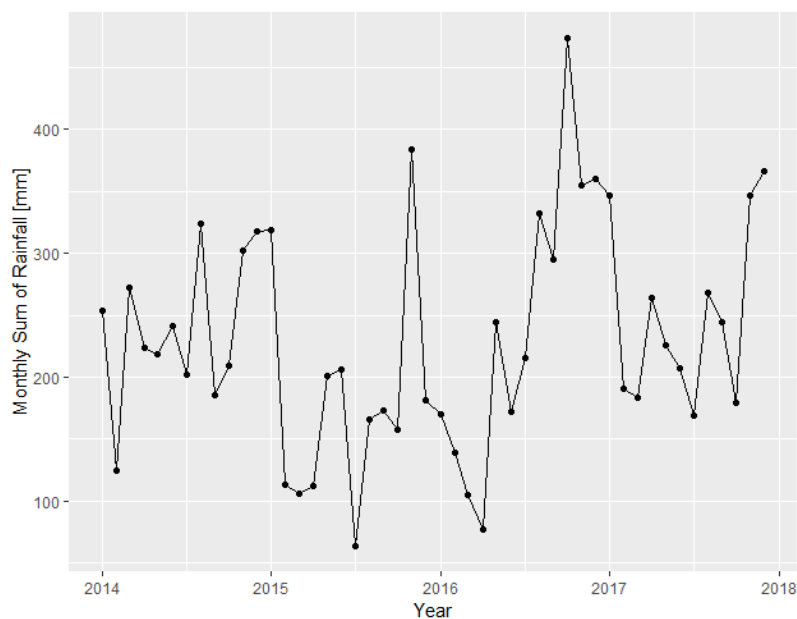


Figure 7. Monthly rainfall data from 2014 until 2017.

Total above ground live tree biomass

The total AGB was significantly higher in primary forest plots compared to the logged forest plots (Table 1; Figure 6) for the year 2015, 2016 and 2018 plots data (ANOVA, $F_{1,16} = 126.6$, $p = 5.19e^{-09}$).

Table 1. The total biomass [t/ha] in the primary forest and logged-over forest. Change in biomass [t/ha] between 2015-2016 and 2016-2018, respectively

Plot	Forest Type	Year	Total Biomass [t/ha]	Biomass Change [t/ha]
1981_1	logged	2015	339.6	0
1988_5	logged	2015	333.1	0
1989_1	logged	2015	242.7	0
Conservation_area_3	primary	2015	917.3	0
Conservation_area_4	primary	2015	788.4	0
Conservation_area_5	primary	2015	824.5	0
1981_1	logged	2016	291.3	-48.3
1988_5	logged	2016	334.8	1.7
1989_1	logged	2016	155.5	-87.3
Conservation_area_3	primary	2016	668.6	-248.6
Conservation_area_4	primary	2016	661.2	-127.2
Conservation_area_5	primary	2016	737.4	-87.1
1981_1	logged	2018	319.3	28.0
1988_5	logged	2018	353.9	19.1
1989_1	logged	2018	149.2	-6.2
Conservation_area_3	primary	2018	662.5	-6.1
Conservation_area_4	primary	2018	730.6	69.4
Conservation_area_5	primary	2018	924.5	187.1

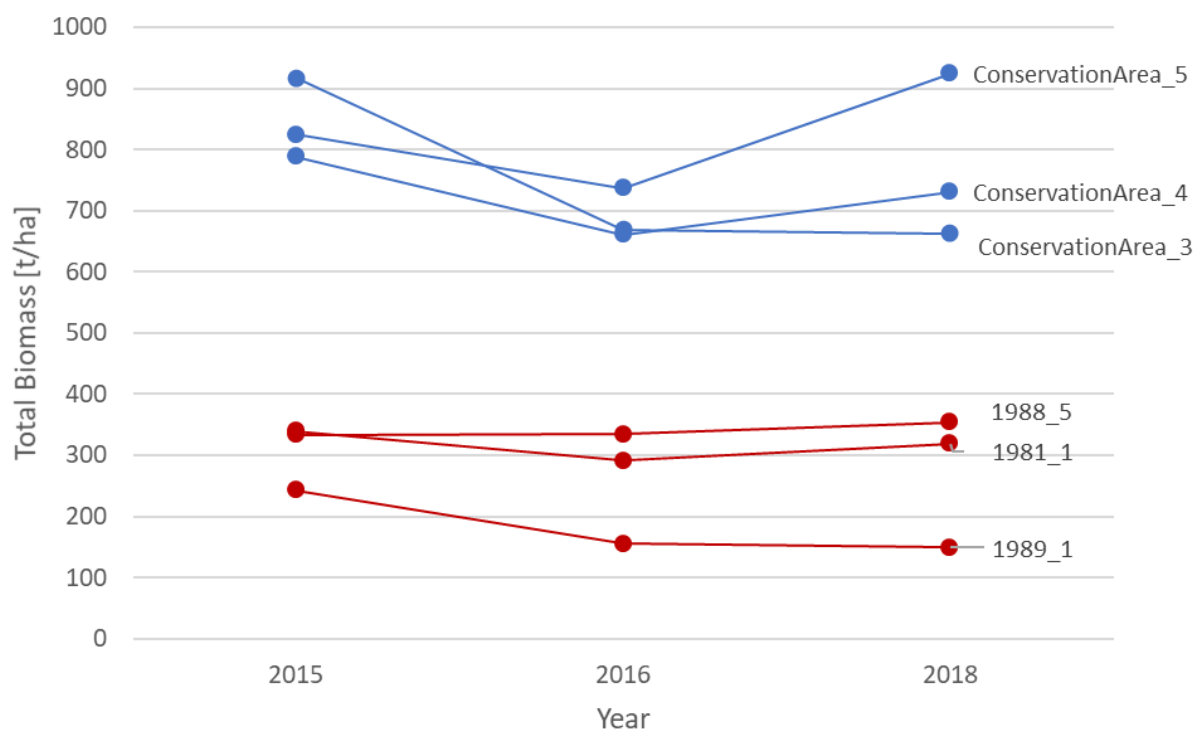


Figure 8. The total biomass of both primary forest and logged forest plots.

Change in biomass related to drought event

Most primary and logged forest plots experienced loss of biomass in 2015-2016 (Table 1; Figure 7) and an increase in biomass in 2016-2018 (Figure 8). Although primary forests had a larger absolute loss in biomass between 2015 and 2016 than logged forests, there was no statistical support for the differences observed (ANOVA, $F_{1,4} = 3.982$, $p = 0.117$). Similarly, no significant differences in biomass changes occurred between logged forest and primary forest for the period 2016-2018 (ANOVA, $F_{1,4} = 1.495$, $p = 0.289$).

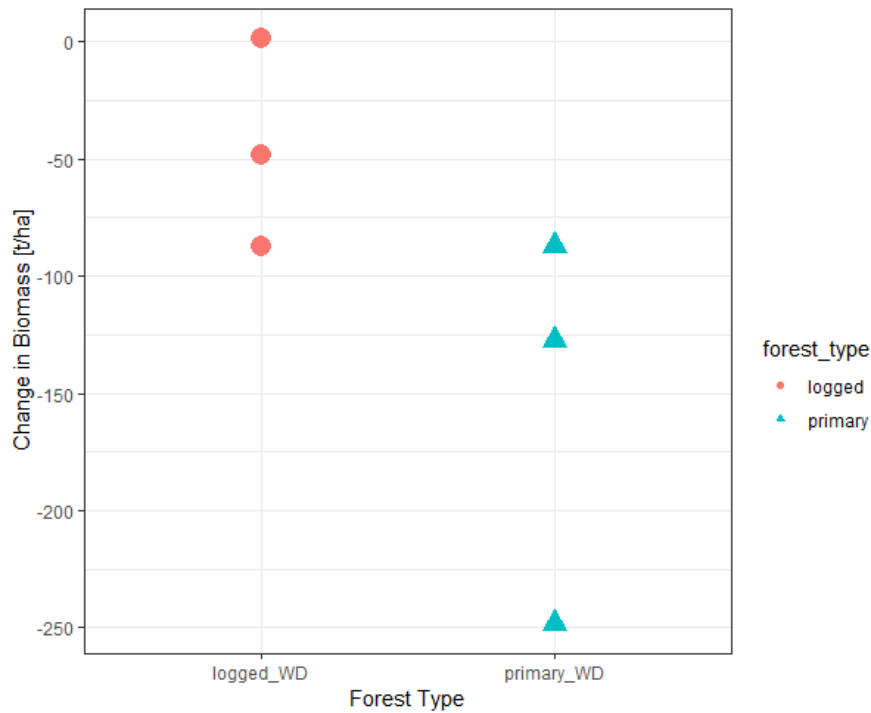


Figure 9. The biomass changes in primary and logged-over forest between 2015-2016

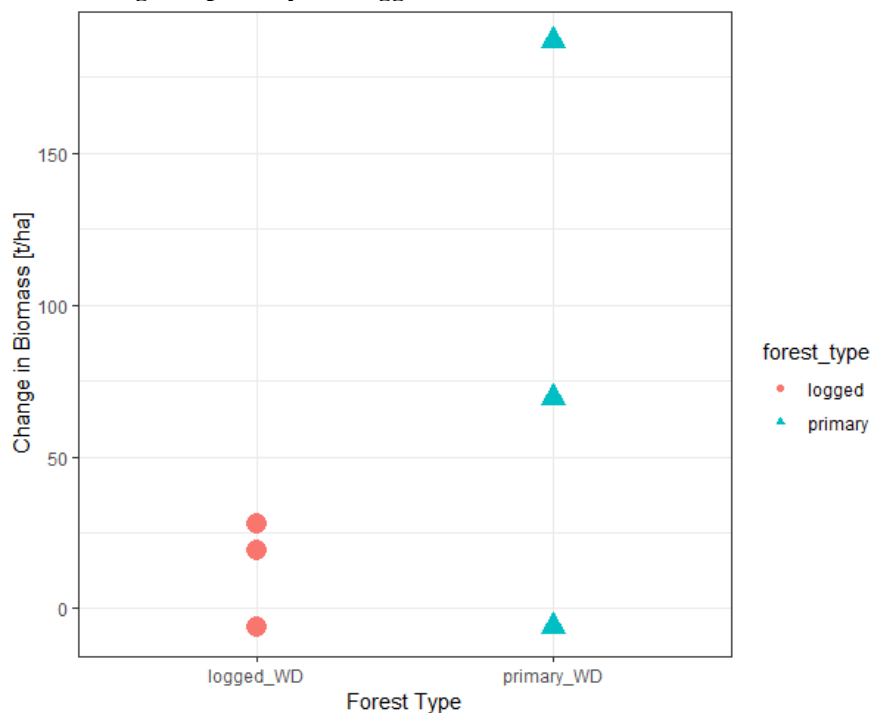


Figure 10. The biomass changes in primary and logged-over forest between 2016-2018.

Forest plots with higher initial biomass (biomass in 2015) seem to suffer more from the drought event and lose more biomass after the drought (Figure 9). However, no significance could be shown with our data (ANOVA, $F_{1,4}=4.8675$, $p = 0.09201$). Interestingly, some high-biomass plots had also the highest recovery rate (Figure 10). Nevertheless, it is difficult to make any suggestion about the effect of high original biomass on the recovery rate after drought (ANOVA, $F_{1,4} = 1.0687$, $p = 0.3596$)

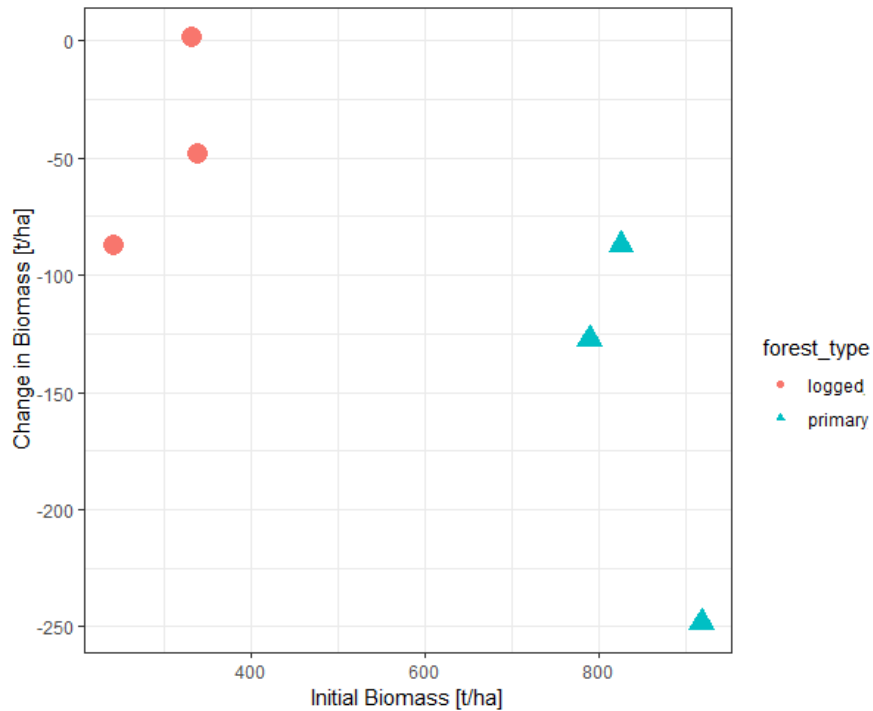


Figure 11. The biomass changes between 2015-2016, related to initial biomass.

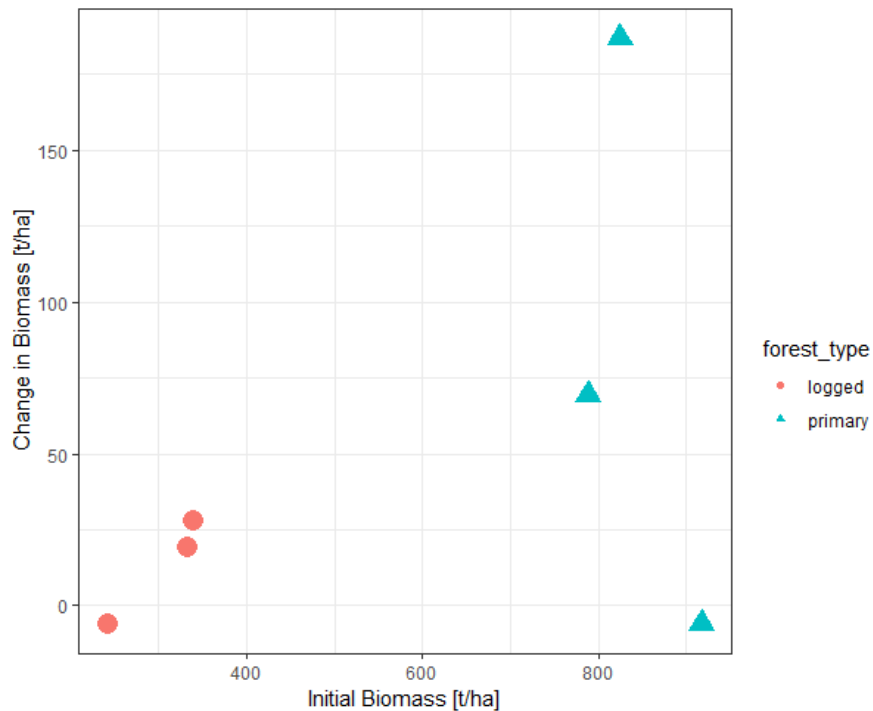


Figure 12. The biomass changes between 2016-2018, related to initial biomass.

DISCUSSION

Climate data

Several months in 2015 and beginning of 2016 experienced lower than average rainfall regime. 2015 fits into the reoccurring five-year pattern of low annual rainfall that could be observed. More serious drought events occurred in both year 1997 and 1985. Walsh (1996) defined drought as months with less than 100 mm rain. But based on the rainfall data from DVCA, we found rainfall in between year 2015 and 2016 fit into the reoccurring five-year patterns with low rainfall and therefore concluded it to be a drought event.

Nevertheless, it might be worth having a closer look at the rainfall patterns for other drought-related studies in this area by including the number of consecutive days with very low rainfall. This was suggested by Mikey O'Brien (Christopher Philipson, pers. comm.), in order to reflect the water availability situation for forest trees. While trees can survive a number of days with no or low rainfall by using the water reservoir in the soil, they do suffer from water shortage after passing a threshold of continuously low rainfall days.

Total above ground live tree biomass

Primary forests have significantly higher total above ground live tree biomass than logged forests. It is suggested that this is mainly due to the presence of large trees that are missing in the logged forest. However, it is wise to keep in mind that in this study, plots of 0.1 ha had been measured and then extrapolated to 1 ha plots. This implies that primary forest plots with more large trees than average tend to overestimate the total biomass.

Change in biomass related to drought event

Primary and logged forests suffered equally from the drought event in 2015, and no statistical difference could be observed between the recovery rate in primary and logged forests. This suggests that both primary and logged-over forests are of comparable importance in providing carbon-related ecosystem services such as carbon storage. Berry *et al.* (2010) however, found that logged-over forest had faster recovery rates and accumulated five times more carbon than primary forest. Hence, such forests provide significant carbon storage over time.

Two plots measured (Conservation_Area_4 and 1981_1) were affected by strong wind events that created big patches of fallen trees. This led to a major loss of tree biomass in those plots during the recovery period 2016-2018 (Table 1). Since our data set comprises of only three plots per forest type, these tree-fall patches have a proportionally large impact on the overall results. The small number of data points also makes it unlikely to obtain any significant result in the statistical analyses. Therefore, we strongly recommend increasing the number of plots for any further study into forest biomass and resilience. This will also help to balance out the effect of large tree falls on the result. Another possible source of inaccuracy is that we only

measured trees that were already in the plot's tree inventory. Although some untagged trees had grown to a size where they should be included in the inventory, we did not measure them except for one case. We encourage updating the tree inventories in each field season to improve accuracy of the modelled biomasses.

CONCLUSION

The findings of this study indicate that there was a drought event in 2015-2016. Both primary forest and logged-over forest were affected by the drought event with loss and recovery of biomass in the 2015-2018 periods. From this study, it can be assumed that the logged-over forest also plays an important role in providing ecosystem services similar to primary forest. In order to improve the biomass estimates, it is suggested to increase the size of each sampling plot as well as to increase the number of study plots. If this study could be extended, it will be able to provide a more solid estimation of the value of logged-over forests towards carbon storage.

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Camouflage success in rainforests: a study case in Danum Valley, Borneo

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Abstract

Camouflage efficiency is an important factor influencing prey-predator interactions and survival success. Selection of coloration in animals seem to be dependent on the environmental conditions found in different habitats. This study tested the detection success of mammals with unicolor, spotted and striped patterns. Participants were asked to spot mammal figures hidden in different vegetation densities during a limited time and restricted movement. Results showed that unicolor pattern was the most successful camouflage strategy. Vegetation density did not influence the detection success of distinct colouration patterns; and high light intensity decreased the detection success of different camouflage strategies. Unicolor pattern might be absorbing light in shady areas while reflecting it in open sites, leading to high camouflage effectiveness. Striped patterns seemed to perform better in open areas, whereas dotted patterns were less effective across all vegetation densities and light intensities.

INTRODUCTION

In wild ecosystems there is a wide range of animal coloration which appears to have several advantages related to different selective pressures (Caro, 2005). Animals that resemble their natural environment (i.e. colour of their background) remain concealed and cryptic. This resemblance can be presented as a uniform coloration (one colour) or different patterns in some body parts (e.g. belly, tail, face) or in the whole body. It is important to highlight that a successful camouflage should favour countershading (ventral parts lighter than dorsal parts) and disruptive coloration (colour patches breaking contour of animals; Gomez & Théry, 2007; Sobroza & Goncalves, 2016).

Some studies have shown that animal coloration depend on the lightness or darkness in the environment (Caro, 2005). For example, pale or soft coloured species might inhabit open environments (high lighting) such as in deserts because it decreases water evaporation; whereas dark coloured species are likely to be found in dense environments (low lighting) such as rainforests as protection against ultraviolet radiation.

Furthermore, patches of colour have been said to be used as communication between and within species (Gomez & Théry, 2007). That is, as a signal of beware or intimidation towards a predator, subordination

within populations or reproductive condition, which is likely to be related with health and genetic quality (Caro, 2005; Gomez & Théry, 2007).

Some examples of communication include tail coloration in ungulates that is related with diurnal activities and social groups. Likewise, white spots in certain parts of the body (ears or tail) in felids has been related with social recognition (i.e. young individuals towards their mothers); while black and white coloration is usually a sign of beware for aggression (Caro, 2005).

Species with a successful coloration are likely to have higher survival rates due to lower susceptibility to predation or higher success in hunting (Sobroza & Goncalves, 2016). Moreover, there is a significant relation between camouflage and distance. That is, camouflage patterns are mostly useful at large distances as they resemble a unique colour patch. Thus, at short distances camouflage is likely to be less efficient as each colour pattern will be distinguished (Gomez & Théry, 2007).

There are several theories about the advantages of light and dark colorations. However, the effectiveness of distinct fur patterns in mammals at different densities of tropical habitats, has not been tested (Caro, 2005; Gomez & Théry, 2007; Sobroza & Goncalves 2016).

In this study we investigated the effectiveness of camouflage by (1) determining detection success of three different coloration patterns (dots, stripes and unicolor), (2) assessment of dependence of camouflage strategies within different vegetation densities and (3) evaluate if light intensity have an influence on detection success of different coloration patterns. Predictions of this study were that (1) unicolor camouflage was going to be a more successful adaptation through all vegetation densities, (2) the detection rate of different camouflage strategies would be dependent on the vegetation density and (3) higher light intensity would increase detection success.

MATERIAL AND METHODS

Study site

This study was carried out in the north-west of Borneo Island (Malaysia). More concretely, in Danum Valley Field Centre located in the Sabah territory. Danum Valley is a conservation area covered by tropical primary forest and some patches of tropical selectively logged forest.

Sampling design

The experiment was conducted in a pergola with four windows (Figure 1). Each window was facing different vegetation densities (Figure 2).



Figure 1. Pergola with four windows (220 x 350 cm) covered by curtains that were opened only during each trial.

Vegetation density in each window was measured using a white A4 paper with a 3 cm squared grid. The coverage was measured counting the number of visible squares once placed on the forest (Figure 3).

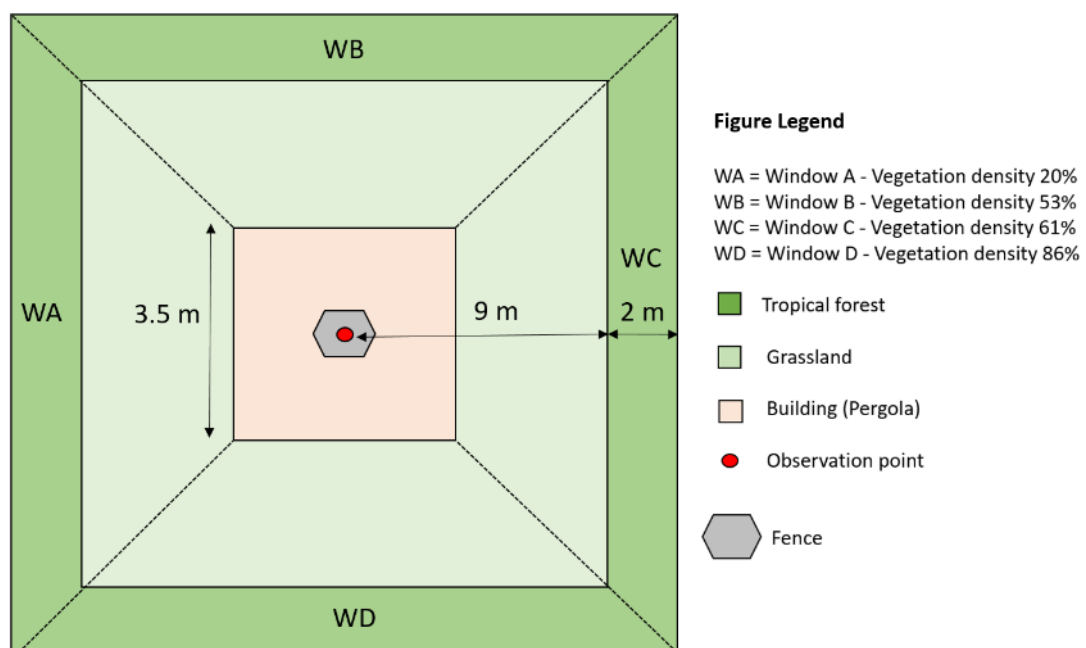


Figure 2. Bird eye view of the study site. Different windows facing to different vegetation densities.

In each window, 12 pictures of the squared grid were taken (4 at the ground level, 4 at 1 m height and 4 at 2 m height from the ground level) with a space of 1 m between them. The vegetation density of the window was obtained by averaging the twelve measurements.

For each trial, a total of 12 cardboard shapes of identical shape were hidden in the tropical forest at a distance of 9-11 m from the observation point (Figure 2). The shape simulated a hidden civet (40 x 38 cm; one of the

most common mammals in the study area belonging to Viverridae family) with three different fur patterns: (1) unicolor - grey colour, (2) dotted pattern - black body and white dots and (3) stripped pattern - black body and white stripes (Figure 4).

Each window contained three hidden shapes (one of each coloration pattern). Hiding locations of the shapes were fixed for the whole experiment (ground level, 1 m and 2 m heights from the ground level). The three patterns were randomly located (for every trial) at three fixed positions and all animal shapes were similarly hidden in the vegetation. Between 40-60 % of the animal's shape was hidden by the vegetation. This was attained using a gridded white civet shape (Figure 4), where white visible squares were counted in pictures taken from the observation point.

Light intensity (Lux) was measured for each participant using a *Three Way Meter*. Three measurements were taken for each player averaging them for subsequent analysis.

Figure 3. Gridded A4 paper with 3 x 3 cm squares. Number of visible white squares determine the vegetation density.



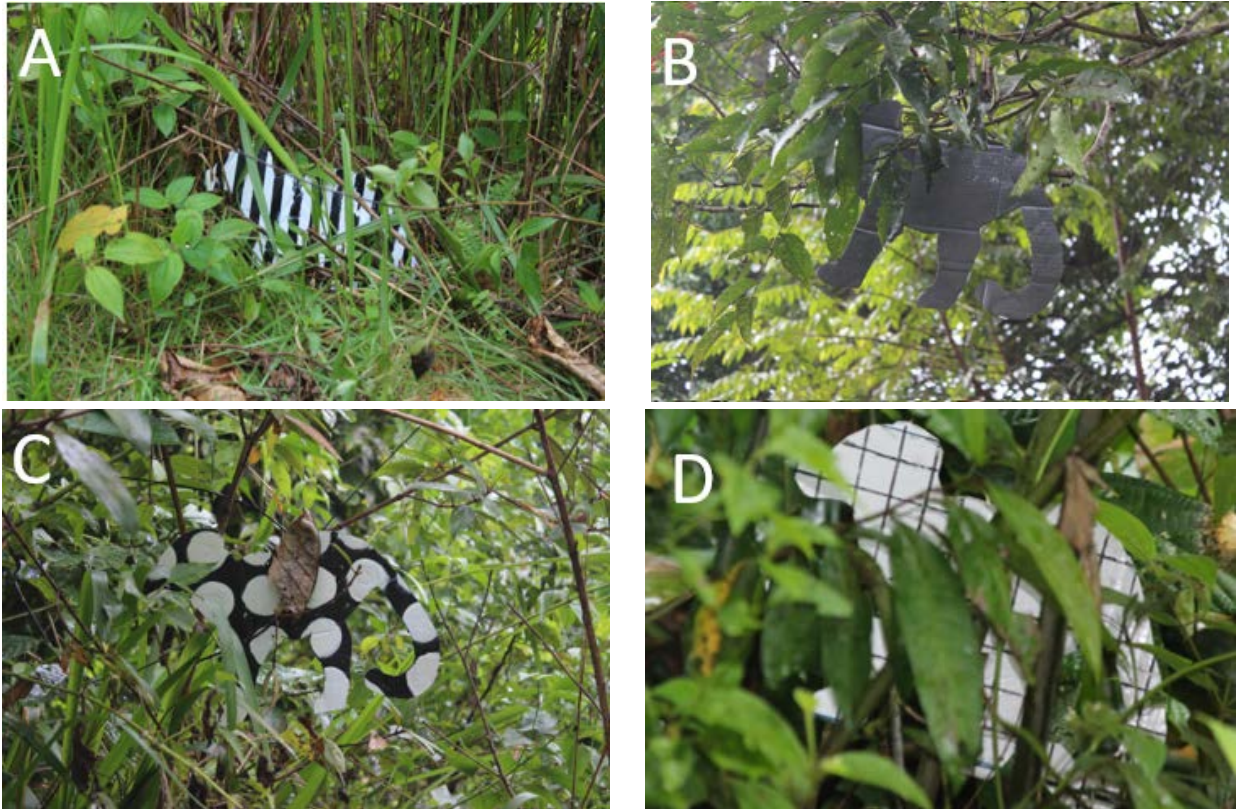


Figure 4. Civet shapes (40 x 34 cm) hidden in the vegetation with different coloration pattern: A. black-white striped; B. grey; C. black-white spotted; D. Civet shape with a 3 cm square grid. Methodology used to ensure a vegetation coverage from 40-60% of the animal shape.

Data collection

The dataset was collected in October 2018. A total of 66 participants were randomly selected to participate in the experiment. Before starting the experiment, personal information such as gender, age, nationality, height and naturalist experience (*i.e.* each participant ranked their own naturalist experience from 0 to 10, with 0 corresponding to no abilities and 10 to an experimented naturalist) were taken for each participant.

They were informed that the game consisted in spotting fake animals in four different windows, with a maximum time of 60 seconds. Each participant was asked to stay at the observation point without moving (Figure 1). The four different windows were randomly opened one at a time. Spotting time of each animal shape was recorded as well as the coloration pattern (*i.e.* unicolor, striped or spotted) and location (ground level, 1 m or 2 m above the ground level). No further information regarding shape, size, pattern or number of targets per window was given to the participants.

Data analysis

Statistical analysis were realized using the software R 3.4.3 (R Development Core Team, 2005). Graphs were developed using the R package ggplot2 (Wickham 2016).

Calculate the detection success of each shape

DetSuc (detection success) was used as response variable in the analysis. This variable was calculated for each shape using the following formula:

$$DetSuc = \frac{\left(S + \frac{(60 - T_s)}{60}\right) * 100}{2}$$

S (success) was a factor indicating if the shape has been detected by the observer (yes = 1, no = 0) while *T_s* (spotting time) was the number of seconds required by the observer to detect the shape. Using the formula, an observer that detected a shape very fast (2 s) obtained a detection success of 98.3% while an observer that required 58 s to detect the shape obtained a detection success of 51.7%. In the case of the observer could not find the shape, he obtained a detection success of 0%.

Coloration patterns

The mean of *DetSuc* values of the camouflage patterns were compared using boxplots and ANOVA. In addition, a rank was attributed (first, second and last) to the shape relatively to their detection time in each window. Bar plots were used to visualise the distribution of these ranks between the three different studied patterns.

Environmental factors

Relationship between the response variable (*DetSuc*) and the environmental variables (light intensity and vegetation cover) was investigated using linear regressions and environmental variables as exploratory variable.

Influence of the coloration patterns on the *DetSuc* in different environmental conditions was tested by performing linear regressions using environmental variables and pattern as exploratory variables.

Participant personal information

Because each experiment encompassed 12 shapes, average of the *DetSuc* values were calculated for each observer. These results were compared by means of different categorised exploratory variables (sex and nationality) using boxplots, t-test and ANOVA. In addition, linear regression was performed to investigate the relationship between *DetSuc* and the numeric exploratory variables (age, sex and naturalist experience).

RESULTS

Environmental factors: Vegetation density, light intensity and coloration patterns

Different coloration patterns showed variation in detection success rates (Figure 5a). Dotted pattern displayed the highest detection success (median = 68.3%) followed by the striped pattern (65.0%) and unicolor pattern (57.1%). Though, there was no statistical evidence to prove that different patterns have different detection success (ANOVA, $F_{2,787} = 2.83$, $p = 0.06$).

Detection success (%) decreased as light intensity (Lux) was increasing. This relation was presented for all patterns following a similar trend (Figure 7). For different light intensities, unicolor pattern constantly experimented the lowest detection success, whereas dotted pattern had the highest (Figure 5b).

Detection success (%) of dotted and unicolor pattern was not influenced by the vegetation cover (%). However, striped shapes experienced a higher detection success with increased vegetation densities, following a positive linear trend ($\beta = 0.05$, CI [-0.05, 0.16]; Figure 5c).

Comparing the detection ranking of different coloration patterns, dotted camouflage was frequently spotted faster while striped pattern was mainly spotted at last position (Figure 6). Detection failure was considerably higher for unicolor pattern ($n = 63$) compared to striped ($n = 30$) and dotted patterns ($n = 34$; Figure 6).

Human factors: Sex and naturalist experience

Detection success was significantly higher ($p < 0.05$) for men (median = 67.6%) than women (56.3%). However, the dataset contained more male observations ($n = 43$) than woman ($n = 23$; Figure 7a).

Participants with higher naturalist experience performed higher detection success (%), following a positive linear trend (lm, $\beta = 1.3$, CI [0.91, 1.70]; Figure 7b).

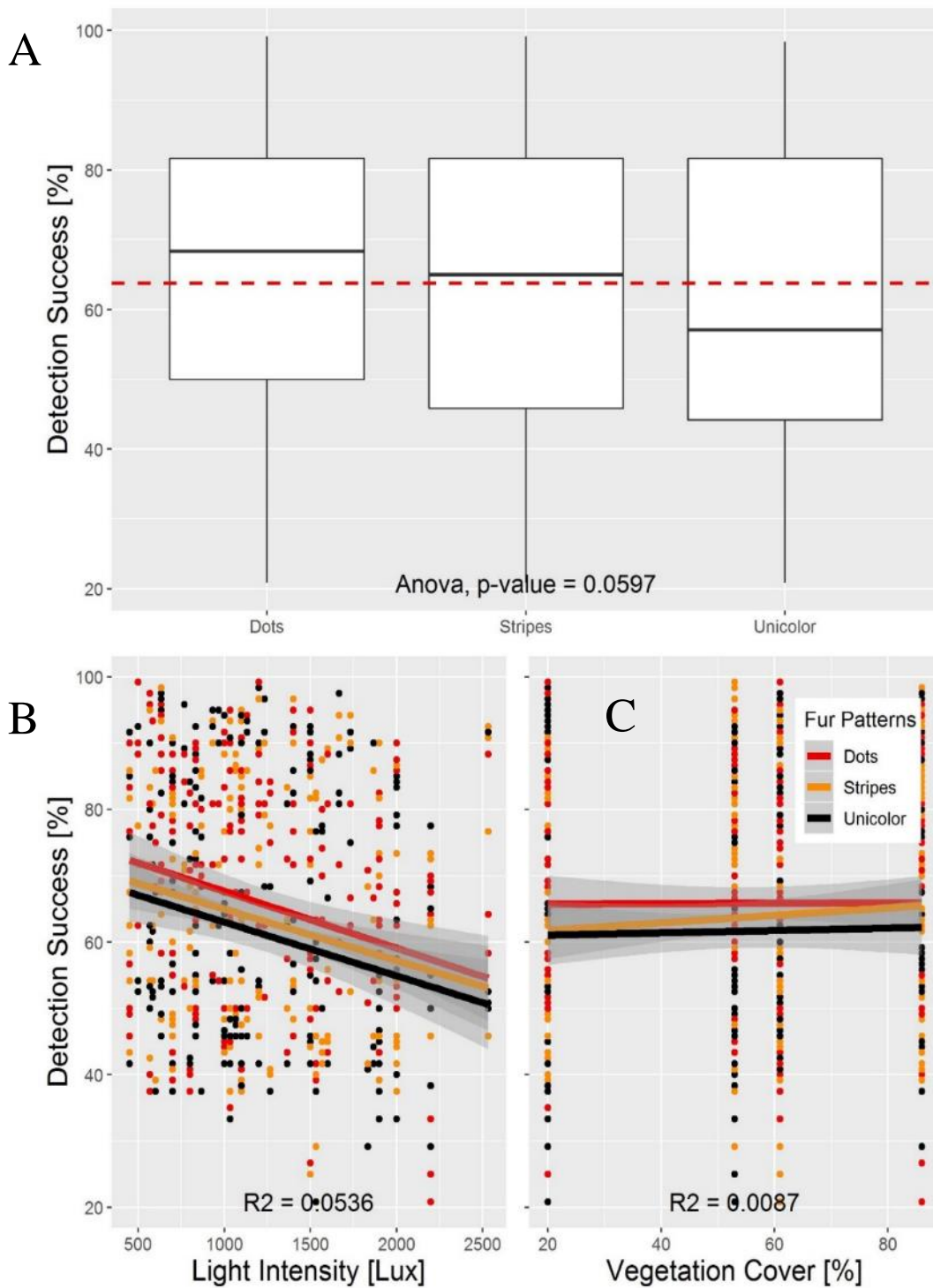


Figure 5. A. Detection success (%) of different patterns (Dots, Stripes and Unicolor). **B.** Detection success (%) of different patterns (red-dots, yellow-stripes and black-unicolor) at different vegetation cover (20%, 53%, 61% and 86%) **C.** Detection success (%) of different patterns (red-dots, yellow-stripes and black-unicolor) at different light intensity (Lux).

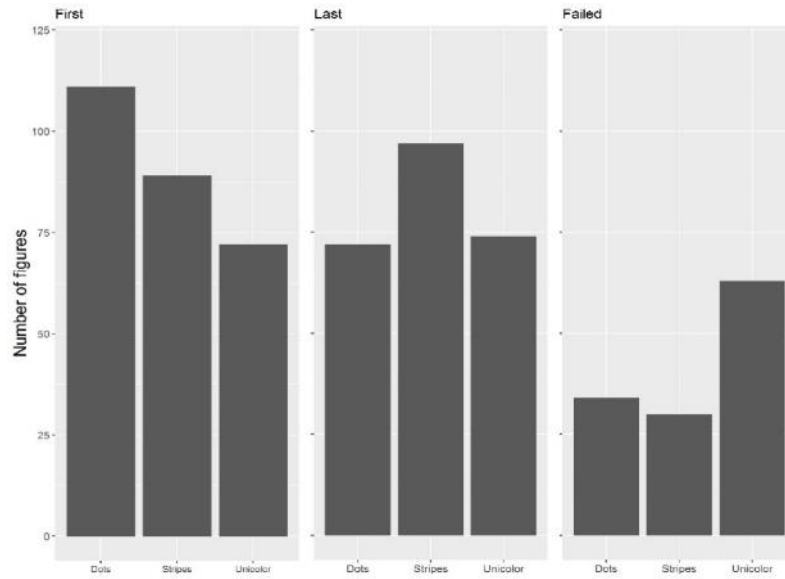


Figure 6. Spotting order and failed detections for each trial. Showing the number of figures (dotted, striped and unicolor) spotted in first, last position and failed detections.

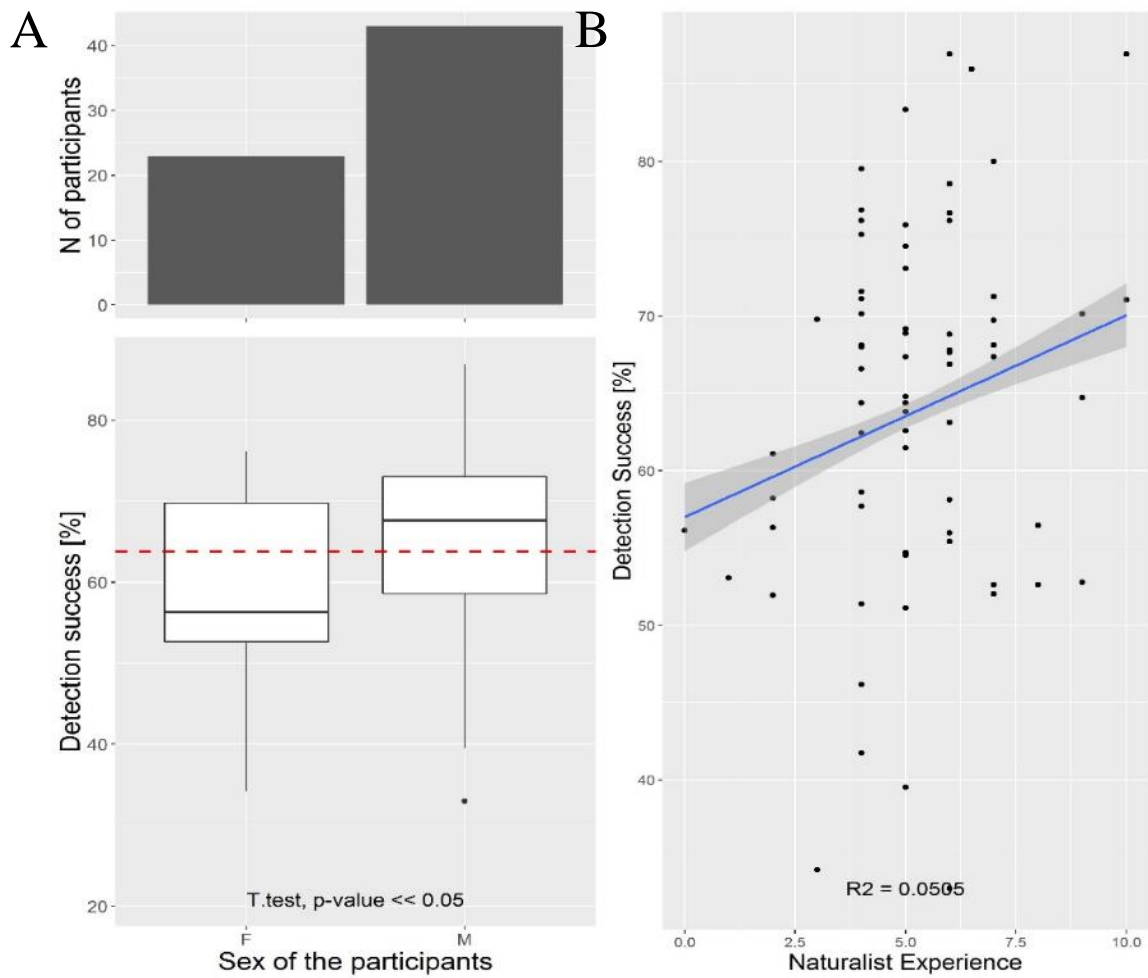


Figure 7. A. Detection success (%) comparing participant sex, B. Detection success (%) plotted against self-evaluation of naturalist experience (i.e. rank your personal naturalist abilities from 0 to 10, being 0 no abilities and 10 an experimented naturalist).

DISCUSSION

Environmental factors: Vegetation density, light intensity and coloration pattern

Camouflage is an efficient strategy for animals to avoid predation attempts or being a successful predator. As expected, a unicolor pattern was the most effective camouflage in different vegetation densities and light intensities in comparison to black-white dotted and black-white striped patterns. However, no significant statistical evidence between each pattern was shown.

These findings are consistent with Caro (2005) and Sobroza *et al.* (2016), which have reported that black-white dotted patterns are not specialised in hiding but communication and prevention. Animals that present these colorations might be trying to avoid predation by warning their predator about some defensive behaviour. However, even when conspicuousness might not be a principal precaution for these animals and the detection speed for it was the fastest, results obtained in this study suggest that vegetation density and light intensity seemed to have certain importance. A simple explanation could be prey-predator interaction. As prey intend to overcome predation, predators have adapted to their defensive behaviours, forcing prey to try to be unnoticed in dense vegetation or bright environments.

Moreover, according to previous studies (Caro, 2005; Gomez & Théry, 2007; Sobroza *et al.*, 2016), there is evidence that unicolor patterns, specifically dark colourations are highly conspicuous in shady and dense environments. However, results in this study showed that high light intensity also offers a high camouflage success. By a way of explanation, as grey colouration (unicolor pattern) is not precisely a dark colour, it could be absorbing the light in shady areas, whereas in open and bright sites, light is likely to be reflected, which results in high camouflage effectiveness, thus low or no detection speed.

Furthermore, black-white striped pattern appeared to be a less effective camouflage in comparison with unicolor coloration, but not when compared to dotted pattern. According to Caro (2005), striped patterns can be mainly found in predators (*i.e.* felids); yet, black-white striped patterns are not common through natural communities. White signals will induce high brightness contrast at different heights, while black signals are not usually processed by colour vision, which might provide efficient disruptive coloration (Gomez & Tréy, 2007). This suggests that black-white striped colouration should be more effective and conspicuous in bright areas, which in fact is consistent with the results of this study. Predators might have a higher hunting success in open environments as light will be reflected in other animals and they often use brightness cues to detect a target at large viewing distances and these distances allow them to implement hunting strategies to corner a prey. To that end, low detection speed might be due to this predator adaptation.

Human factors: Sex and naturalist experience

Environmental factors are not the only variables influencing the effectiveness of a camouflage pattern. The gender of each participant showed influence when classifying the camouflage success of a coloration pattern.

Results showed that men displayed a significant higher detection success than women. However, it is important to highlight that the number of participants were mostly men. Men detection success might be related to an evolutionary process that includes past occupations: men being hunters and women gatherers. Moreover, the naturalist experience of participants is related with their nationality (see Appendix), as most of them were Malaysian and experimented research assistants in tropical rainforests.

Additionally, there was an influence of learning and memorising the coloration patterns and positions (heights) of the shapes while spotting at them. These results might have had an influence in the detection success of participants and subsequent analysis (see Appendix).

The coloration selected to generate the different patterns may be a limitation in this study as black-white spotted and stripped patterns are not commonly found in tropical native species. Moreover, due to time restrictions the study had a limited sample size.

Future studies should include the reproduction of this experiment using different colour patterns commonly found in tropical forests such as combinations of black with brown, grey or yellow. Another interesting approach would be to test different animal shapes and sizes, targeting distances or even to reproduce sounds or movement. Further analysis such as Principal Component Analysis (PCA) could reveal the importance of certain factors on the observation process and camouflage success.

In conclusion, the detection success of three different coloration patterns (dots, stripes and unicolor) and the assessment of dependence of camouflage strategies within different vegetation densities and lighting densities was successfully tested. According with the predicted hypothesis, unicolor camouflage was successfully adapted through all vegetation densities. Though detection rate of different camouflage strategies was not dependent on the vegetation density as well as higher light intensity did not increase detection success.

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APPENDIX

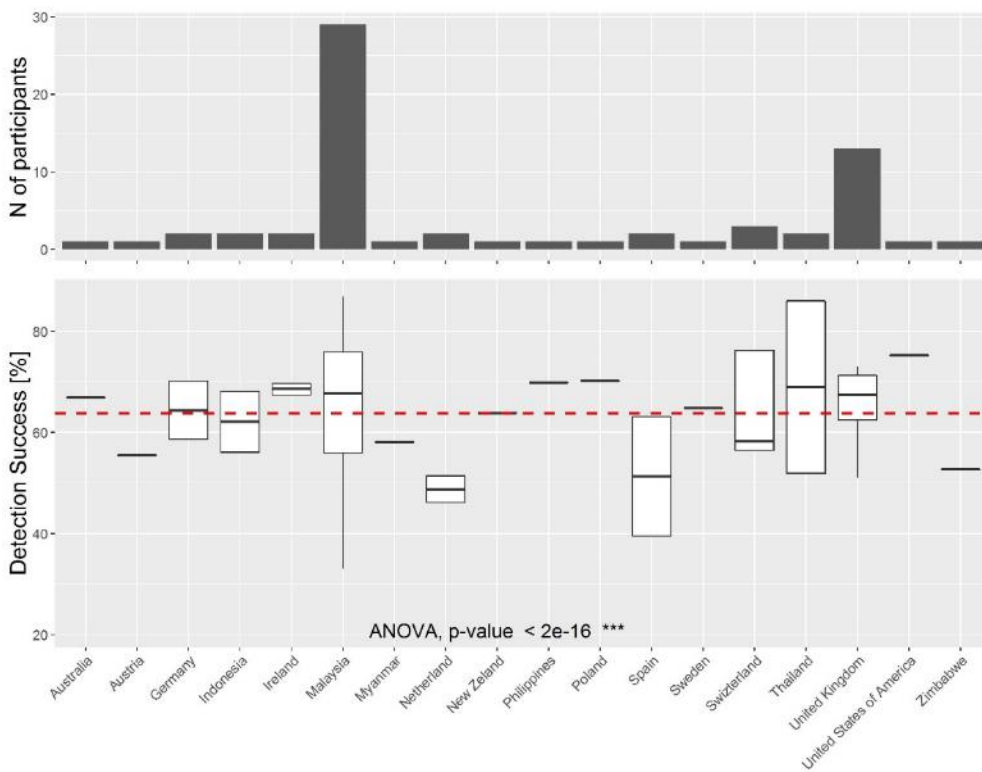


Figure 9. Detection success (%) according to the nationality of the participants.

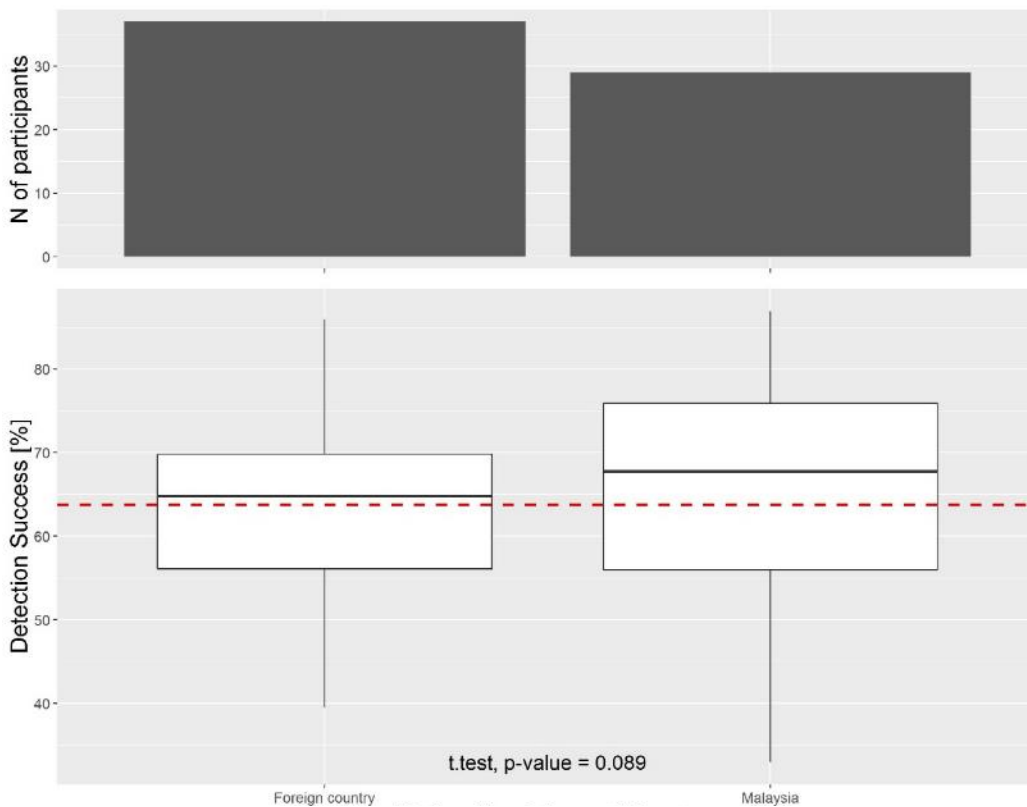


Figure 10. Detection success (%) in relation to two nationalities: Malaysia and foreign countries.

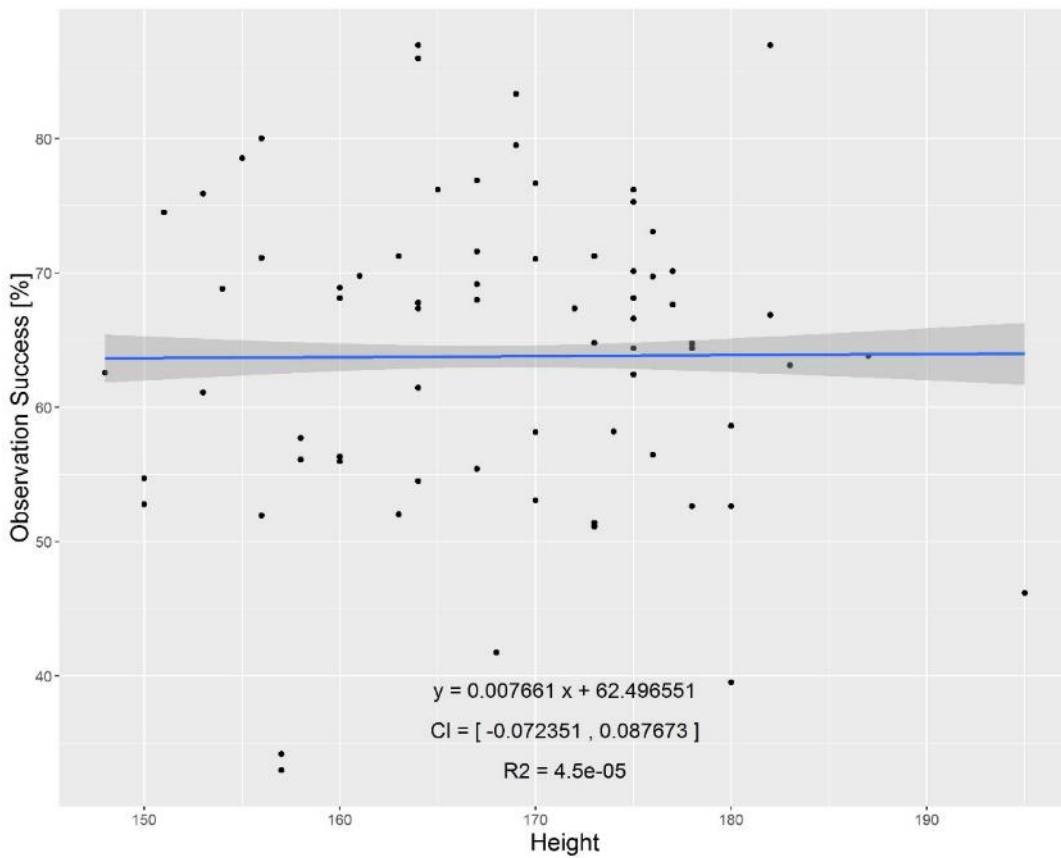


Figure 11. Detection success (%) according to the height of the participants.

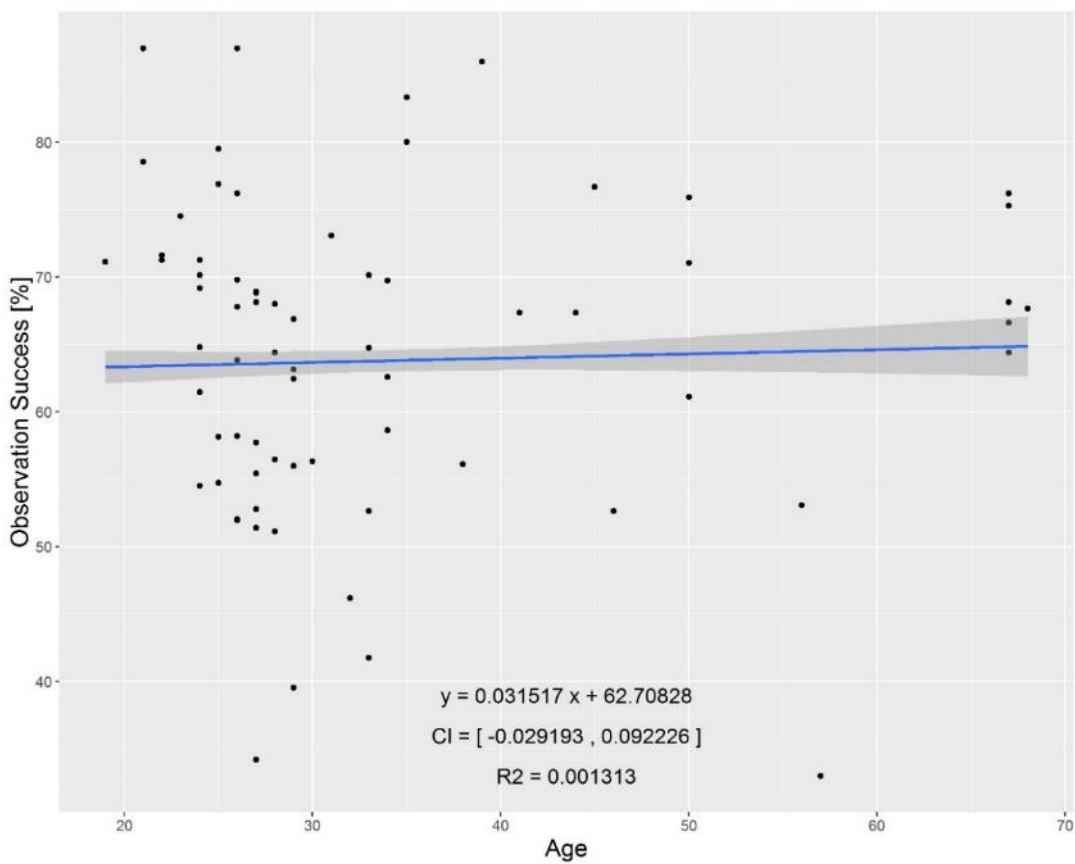


Figure 12. Detection success (%) according to the age of the participants.

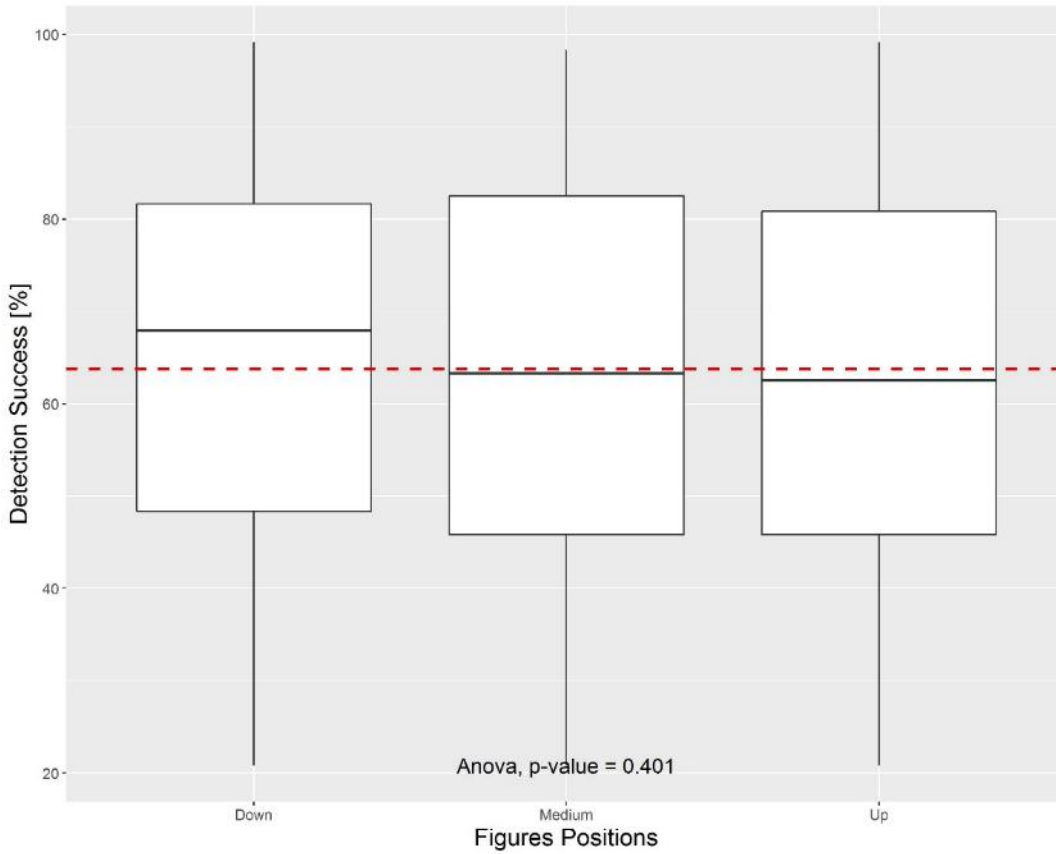


Figure 13. Detection success (%) in relation to the position of the figure.

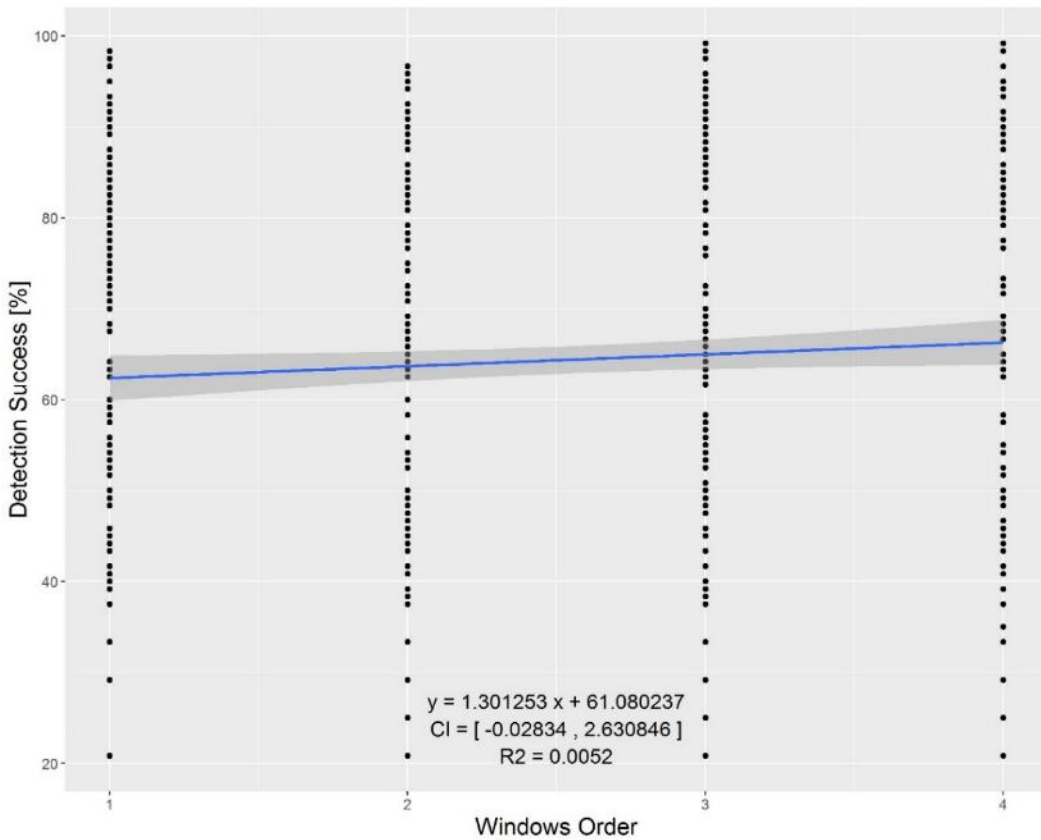


Figure 14. Detection success (%) in relation to the windows order during the experiment.

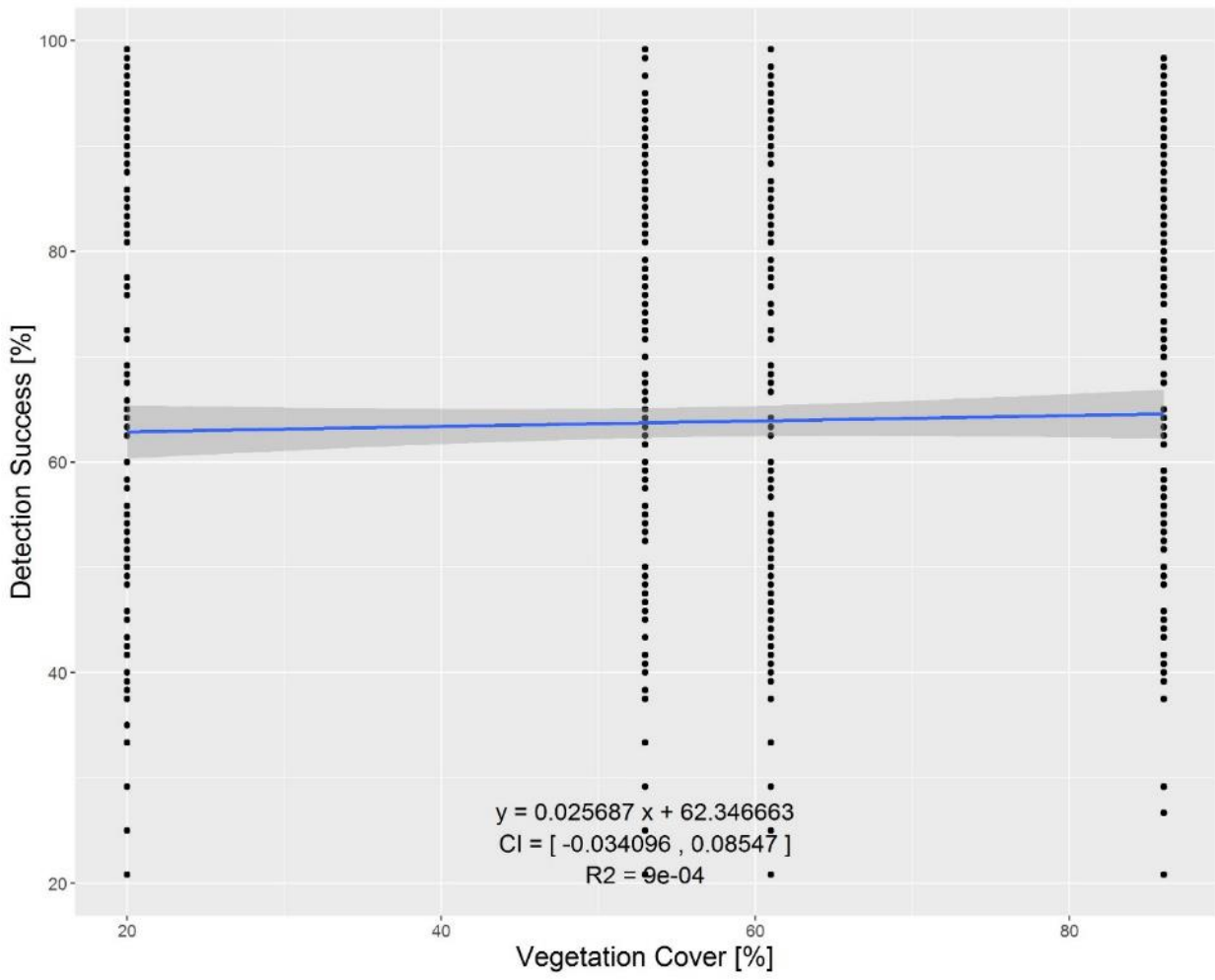


Figure 15. Detection success (%) according to the mean vegetation cover for the three patterns.