Amphibian diversity in Bornean forests: a comparison of habitats using different sampling methods and richness estimators.

*Polypedates colletti* in kerangas habitat (Picture by Emily Waddell)

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Abstract

In light of the global amphibian declines there is an urgent need for baseline surveys in areas, previously unstudied in order to assess geographical distributions and population densities. Population trends cannot be assessed if these surveys are not carried out. There is a special need for such studies in many parts of the tropics where little research has been done and the potential for new species is high. Indonesian Borneo is an area of the tropics where few amphibian surveys have been done and little is known about what species occur there. This study was carried out in Bawan forest, an unprotected and unstudied area of tropical rainforest in Central Kalimantan of Indonesian Borneo. Five different forest types were compared in terms of their amphibian diversity. Visual encounter survey and MacKinnon’s List methods were compared to assess which was better for rapid assessment of the area.

The results showed that the third habitat, a heath forest with a stream, was the most diverse whereas the most disturbed habitat, a newly burnt area, was the least. MacKinnon’s List method was shown to be slightly better than VES yielding two more species. However this technique was biased against groups of individuals and it cannot produce viable relative abundance calculations or species diversity indices. Overall, it was concluded that MacKinnon’s List method as a technique to survey tropical amphibians is advantageous as it uses the time in the field more efficiently and opportunistic data can be included so it does not waste any data and inexperienced field workers can use it. These results along with other flora and fauna surveys should help Bawan forest gain protection from illegal loggers and coal miners.
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Introduction

Amphibian Declines

Vertebrates are currently facing the highest extinction rates in what is arguably the largest mass extinction in history (Cushman, 2006; Gardner et al., 2007). Within this group the amphibians are one of the worst affected with approximately 42% of species in decline and around one third in the three highest IUCN categories (Vulnerable, Endangered and Critically Endangered) (IUCN, 2008). 7.4% of species are close to extinction (critically endangered) compared to 3.8% of mammals and 1.8% of birds (IUCN, 2008). More worrying 22.5% are Data Deficient compared to 5.3% of mammals and 0.8% of birds, which means the current numbers of species in decline is likely to be underestimated (Gascon et al., 2007). For many amphibian species no population surveys have been carried out so there are no baseline data for comparison with future surveys. It is also very difficult to prove full scale extinction and the current figure of 34 species since 1500 is grossly underestimated (Stuart et al., 2004). The reasons for amphibian declines are understood in certain areas where over-exploitation or habitat reduction is clearly to blame (Stuart et al., 2004). However, there are areas where populations appear to be declining despite the habitat being pristine and no exploitation occurring. These declines are due to so-called ‘enigmatic’ causes, most likely climate change and disease (Chytrid fungus and viruses) (Stuart, et al., 2004). Herpetologists became aware of amphibian declines in the late 1980s and several years later in the early 1990s the first Global Amphibian Assessment was carried out to understand the declines and figure out how to mitigate the causes (Stuart et al., 2004).
Borneo

Borneo is the third largest island in the world covering 0.2% of Earth’s surface (MacKinnon et al., 1996). Indonesia, which includes most of Borneo, is one of the world’s 17 megadiverse countries for both species numbers and endemism (Mittermeier and Mittermeier, 1997; Iskandar and Erdelen, 2006). There is a vast range of habitats found on the island including mangrove, peat swamp, lowland dipterocarp forest, heath (kerangas) forest, limestone caves, montane forests and plantations (MacKinnon et al., 1996). These habitats are home to some of the world’s most charismatic and well studied animals. The Bornean orang-utan (*Pongo pygmaeus*), Bornean southern gibbon (*Hylobates albibarbis*) and proboscis monkey (*Nasalis larvatus*) are all endemic to the island (MacKinnon et al., 1996). Other groups of animals have not been studied in as much depth and there is great opportunity to discover new taxa, especially in the dense rainforest of Central Kalimantan (WWF, 2010). Amphibians are very understudied in Indonesian Borneo despite the fact that Indonesia is the fifth most diverse country in the world for amphibians (Convention on Biological Diversity, 2010) with 65% of all amphibians found in Borneo, including two genera *Ingerana* and *Meristogenys*, being endemic to the island (Inger and Stuebing, 1997; Inger, 1999; Frost, 2000; Iskandar and Colijn, 2000). Inger and Stuebing have done work in Borneo, mainly in the Malaysian part, on the distribution of species. However, little is known about the ecology, reproduction, biogeographic patterns and evolution of many, if not most species (Iskandar and Erdelen, 2006). This presents a problem when trying to assess the current status of amphibian populations in Borneo, as there are very few previous data for comparison with new data (Iskandar and Erdelen, 2006).
The Orangutan Tropical Peatland Project (OuTrop) is an organisation based in Central Kalimantan, Borneo. The research it carries out is primarily on the primates found in Sebangau National Park, though they also carry out other biodiversity surveys. As the area has been logged and burned in previous years, the main aims of the project are to monitor and protect the remaining forest and promote forest generation in the disturbed areas (OuTrop and CIMTROP, 2010).

Threats to the fauna of Borneo

Bornean wildlife is highly threatened and with large numbers of endemic species, which normally occupy limited geographical ranges, extinctions are probable (Iskandar and Erdelen, 2006). Most of the endemic species in Borneo live in forests (Iskandar and Erdelen, 2006). The main threats are forest degradation and deforestation (FAO, 2010). The huge worldwide demand for palm oil has increased the creation of palm oil plantations and has become the leading driver of deforestation in south-east Asia. Palm oil is a very useful crop to many as it is the ingredient in food products, cosmetics, engine lubricants and biodiesel. Therefore it is of huge economic importance to the countries that cultivate it (Butler et al., 2008). In Indonesia and Malaysia palm oil production has increased greatly in recent years, resulting in Indonesia becoming the second largest emitter of greenhouse gases in the world due to deforestation (Butler et al., 2007). The rare peat swamp forests, which are home to the critically endangered Bornean orang-utan, have decreased greatly in recent years due to the creation of plantations (Fitzherbert et al., 2008; Venter et al., 2009). It is not just mammals which are badly affected. Studies show
that no forest dwelling amphibians occur in palm oil plantations suggesting that they cannot adapt to this extreme habitat change (Iskandar and Erdelen, 2006).

Mining and selective logging results in forest degradation which can greatly alter the habitat affecting the fauna within (Butler, 2008). Gold mining involves using mercury which gets into river systems, killing fish, altering the river ecosystem and affecting local communities (Butler, 2008). Selective logging involves the removal of high quality hardwoods which opens up the forest increasing its susceptibility to fire (Butler, 2006). The building of roads and the subsequent pollution from both these industries leads to further deforestation and forest degradation respectively (Butler, 2006).

Climate change is another major factor affecting the forests of Borneo. The El Niño Southern Oscillation (ENSO) is an event when the ocean currents in the Pacific change, causing differences in the climate (Solomon et al., 2007). These are occurring more frequently causing the dry season in Borneo to be drier and longer, increasing the chances of forest fires (Solomon et al., 2007). In peat swamp and heath forests, when the peat layer is lost, regeneration is virtually impossible, leading to the loss of habitat for many species (Janzen, 1974; MacKinnon et al., 1996). Prolonged dry seasons may cause disturbances in amphibian reproductive patterns which may affect populations in the long term (Bickford, 1998). Observations in Papua New Guinea showed that drought conditions affect frogs with terrestrial breeding modes and with direct development (e.g. Philautus spp. found in Borneo) to the point that reproduction almost ceased (Bickford, 1998).
It was previously predicted that by 2010 Kalimantan would have lost all of its lowland forest (Iskandar and Erdelen, 2006). Fortunately this is not the case. However more than 500,000 ha of forest (0.067% of Borneo) are still being lost each year in Borneo, one of the highest rates in the world (FAO, 2010). The Convention on Biological Diversity has highlighted that it is vital to conserve the forest of Indonesian Borneo due to the high numbers of threatened and endangered animals found within (Convention on Biological Diversity, 2010). Perhaps more importantly is the fact that most taxa have not been properly described in this part of the world, meaning many species may become extinct before they are discovered by science (WWF, 2010).

**Visual Encounter Survey**

The Visual Encounter Survey (VES) method is used to produce a species list for an area, to determine the species richness and estimate relative abundance (Crump and Scott, 1994). However, the method cannot determine densities unless combined with a mark-recapture study (Donnelly, 1989). Procedure for a VES involves searching a habitat, either along a transect or in a plot, and recording the number of individuals encountered per unit of time (person hours) (Crump and Scott, 1994). VES generally requires minimal equipment: data sheets, pencil/pen, callipers and spring balance (if measurements are taken), plastic bag and marker pen (if individuals are to be collected) (Crump and Scott, 1994). Sticks or rakes may also be used to turn over leaves and small logs (Crump and Scott, 1994). The VES works best for surveying forest understorey anurans that are active in the open (Crump and Scott, 1994). It is also the best method for surveying rare species or those which are unlikely to be caught with traps (Crump and Scott, 1994). However
this technique cannot be used to survey fossorial species or those that live in the canopy (Crump and Scott, 1994). To avoid biases between individuals, each person must have had the same training, be at the same level of expertise and put the same effort in when surveying (Crump and Scott, 1994). Another bias with the VES is that not all habitat types and microhabitats within a habitat can be sampled with equal success due to differences in strata (Crump and Scott, 1994). Open habitats can be surveyed more efficiently than those with dense vegetation as visibility is better (Crump and Scott, 1994). Weather can also affect visibility, with rain and mist generally decreasing it (Crump and Scott, 1994).

MacKinnon’s List Method

MacKinnon’s List is a rapid assessment method for surveying avifauna which was first suggested by MacKinnon and Phillips in 1993 (O’Dea et al., 2004). It is an effective way to create species discovery curves in order to estimate the total number of species inhabiting a specific area (Bibby et al., 1998). The method also provides a way to calculate an index of relative abundance between species within an area as well as within species between areas (Bibby et al. 1998). Although typically used to survey avifauna it has been suggested that this method is also effective when assessing amphibian populations (Muir, unpublished). Muir’s results show that the MacKinnon’s List technique outperforms the traditional Visual Encounter Survey technique when assessing species richness, and indicating when an area is adequately surveyed. The advantage of this method is that data collection is simple and can therefore be carried out by an inexperienced observer, as it is unaffected by skill and concentration (Bibby et al., 1998).
It allows opportunistic sightings to be standardised in terms of sampling effort and included in the lists and therefore used in the analysis. This is a useful tool as there are usually a lot of opportunistically encountered individuals when surveying forests for amphibians. However, this method can be biased against certain species which are difficult to detect which leads to an under-estimation of these species. The converse is also true, especially when including vocalizations. As the study progresses the observer learns more calls which will bias the results as those at the beginning may be missed.

**Aims and Hypothesis**

The aims and objectives of this project are:

- To assess the overall species diversity and abundance of amphibians in the Bawan forest.
- To assess whether there is a difference in diversity of amphibians between the different habitats in the Bawan forest.
- To assess whether VES or MacKinnon’s List methods works better for surveying an area.
- To carry out work that would help protect Bawan forest from logging and mining.
Methods

Study site

Data collection took place in Bawan forest, an area of primary rainforest of unknown size, never before surveyed for amphibians. The area is situated around 65km north of Palangkaraya in Central Kalimantan, Indonesian Borneo. Access to the forest is gained through a 45 minute car journey along old logging roads from Bawan village (Figure 2.1).

Figure 2.1: Top right – map of Borneo, with Palangkaraya marked. Bottom left – map showing the distance of Bawan village from Palangkaraya and the study site (Pondok Bawan) from Bawan village (Googlemaps, 2010).
The project was carried out as part of a biodiversity survey by The Orangutan Tropical Peatland Project in conjunction with the Centre for the International Cooperation in Management of Tropical Peatlands (CIMTROP). The forest has previously been heavily logged and the forest in the area closest to Bawan village has been completely destroyed due to fire. The project as a whole aims to assess the biodiversity of the remaining pristine forest and promote its ecological importance in order for it to gain some form of protection in the future.

Within the forest there are many forest types including heath or ‘kerangas’ (meaning ‘land that cannot grow rice’ in Iban language: MacKinnon et al., 1996; Proctor et al., 1983), mixed peat swamp, low interior and burnt, disturbed forest. The forest is made up mainly of good quality kerangas forest which has many large dipterocarp species and a tall canopy. Six study sites of different habitats were chosen where Visual Encounter Surveys were carried out; kerangas, mixed peat swamp, hilly kerangas with a stream, newly burnt forest, regenerating burnt forest and low interior forest.

**Habitat descriptions**

When cutting the transects through each habitat they were all given different names to make labelling easier. The names given appear in brackets in the text after the habitat type.

The kerangas forest transect (Frog) ran off the main TA transect at 480 metres bearing north, as seen in figure 2.2. This habitat had a shallow peat layer of 10.7cm on average
data from peat depth survey) with a silica-rich soil beneath the peat. It was around 40 metres from the nearest stream (measured with tape measure) and while ephemeral pools can form after heavy rains, they were not present during the ten surveying days. All five transects were mildly undulating due to tree roots and the paths were blocked once or twice each transect due to large old fallen trees. No new trees fell during the entire surveying period of two months. As the forest was good quality kerangas there were several large dipterocarp species with buttresses found throughout all transects and the canopy height was tall with most trees 15-20 metres and some 30 metres (height estimated by local guides). The leaf litter was relatively shallow and there was little undergrowth.

The mixed-peat swamp forest transects (Toad) ran off the main TA transect at 700 metres bearing north, as seen in figure 2.2. This habitat had a deeper peat layer of 55.3cm on average and the trees had more buttresses and aerial roots (pneumatophores). It was around 150 metres from the nearest stream. There were around five permanent pools (two large ones at the start of transect one) as well as several ephemeral ones. The transects were much more swampy and wet than the kerangas transects. The terrain was flat though it mildly undulated due to roots. The canopy was relatively uniform at around 20 metres. The trees were very unstable due to their shallow roots and would often fall over in wet and windy weather. At least 20 trees fell down through the course of the study, causing the canopy to be broken at the start of transect three where several trees had fallen down in the same area.
The kerangas with a stream transect (Kodok) ran off the TB transect at 450 metres bearing north-west, as seen in figure 2.2. This habitat had a shallow peat layer of 11.5cm on average with a silica-rich soil beneath. A stream ran through every transect once and twice on the fifth transect. There were several permanent pools next to the stream and one on transect one under an uprooted tree. There were a few felled trees throughout the transects and the forest edge was met at the end of transects one and three and the start of two and four. Most of the transects were through good quality kerangas with many large dipterocarp species with large buttresses. The canopy height is tall with most trees 15-20 metres and some over 30 metres. At the forest edge the canopy was almost completely gone and the vegetation was made up of mainly *Macaranga* species amongst burnt trunks and fallen logs. The leaf litter was relatively shallow and there was little undergrowth except for grasses around streams and pools. The terrain sloped down towards the stream which made it quite hilly compared with other transects.

The newly burnt transect (Katak) ran along the main TA transect from 180-380 metres then down TC transect 300 metres, as shown in figure 2.2. The peat layer in this habitat had almost gone along TA (5cm at the end) and had completely gone along TC, where it was replaced with a sand charcoal mix. The closest stream was approximately 30 metres from the end (TC 300 metres). There were two permanent pools one just off TA and one on TC. Both were black from the charcoal and had fallen branches in them. There were several ephemeral pools towards the end of TC, after heavy rain. Throughout the transect there were many fallen burnt trees and branches. Some burnt trunks were still standing and only one or two were still alive. Little regeneration had occurred with some pioneer
species growing, mainly grasses and sedges. The terrain was flat (apart from sloping down towards the stream) although it was constantly blocked by many large logs.

The regenerating burnt transect (Zaba) ran along the road in front of camp, as shown in figure 2.2. The peat layer had completely gone and the sand layer was exposed. The transect was around 2.5 metres across, though at parts it widened where the area had been cleared further to cut logs (for the camp) and some wood and sawdust remained. At the edges the vegetation was very thick, grasses, sedges and ferns, and was near impossible to penetrate. There were a few burnt logs amongst the vegetation and a few small trees of about 4 metres in height at the edges. The transect was approximately 100 metres from the nearest stream (camp stream) though from 250 metres onwards the road was very wet and resembled one large connecting puddle. The terrain was flat apart from obvious tyre marks in the sand from motorbikes and cars.

The low interior forest transect (Kumaka) ran along the main TA transect from 900 to 1400 metres, as shown in figure 2.2. This habitat had a deep peat layer of ~100cm on average. The distance from the nearest stream could not be calculated due to the denseness of the habitat. Ephemeral pools formed between hummocks after rain which made the ground swampy. The terrain undulated due to very large hummocks and aerial roots. The forest was dense with small trees of ~20cm in diameter and a low canopy of 15 metres. The usual transect layout seen in figure 2.3 could not be used due to the denseness of this forest.
Figure 2.2: Transect map showing the different habitats and the locations of all transects and stream quadrats.

Legend:

- = transect
x = distance along transect
T = transect number
* = fast stream quadrat
. = slow stream quadrat
≈ = stream

- = regenerated burnt forest

Kerangas forest

Regenerating burnt forest

Mixed-peat swamp forest

Low interior forest

Zaba

Forest edge

Kerangas forest

 Newly burnt forest

Katak

N

W

E

S
**Study duration**

The first two habitats, kerangas and peat-swamp, were each surveyed for ten nights and three days, and the third habitat, kerangas with a stream, was surveyed for seven nights and three days. The fourth and fifth habitats, newly burnt and regenerating burnt, were both surveyed for four nights and the sixth habitat, low interior, was surveyed for two nights, giving a total VES surveying time of 37 nights and nine days. A total of 20 stream quadrats were carried out over a period of seven days. The work was carried out from the 11th of July to the 1st of September 2010.

**Sampling method and species identification**

Each amphibian encountered was caught by hand, placed in a resealable plastic bag, weighed using a spring balance to the nearest 1g and snout vent length measured using dial callipers to the nearest 0.1mm. Individuals were identified in the field using Inger and Stuebing (2005) and were photographed if they were a new species or an unknown species. The identities were confirmed using photographs from frogsofborneo.com and photographs of those individuals still unknown were sent to various experts once out of the field, in order to confirm the correct identity. Once out of the field Latin names were checked using Amphibian Species of the World: an Online Reference (Frost, 2010) to ensure the most up-to-date nomenclature. Individuals which escaped were only included if their identity was 100% confirmed. This was common for tree frogs and those found in and around streams.
Visual Encounter Surveys

The Visual Encounter Survey (Crump and Scott, 1994) was established at each habitat using five 100 metre long parallel transects separated by 20 metres intercepts, as shown in figure 2.3. A narrow path was cut along each transect using machetes, to clear undergrowth, and trees were marked every ten metres to facilitate sampling in the night. Nocturnal walks of the transects were conducted during the data collection period using head torches. Day transects were carried out to establish whether there were any diurnal species. Either four or five persons surveyed during each transect with one or two experienced personnel, two volunteers plus one local guide. The volunteers were at first not familiar with practising the field techniques but as the survey continued they became more experienced with spotting, catching, handling and measuring the amphibians. Low impact visual searches were carried out in which the area was visually searched using sticks to turn over leaves, causing minimal disruption. The searches were carried out two metres either side of the path and up to 2.5 metres in height. During each search the lead person searched in front and at ground level, the next two persons searched either left or right and the fourth person looked upwards. At the start of each VES air temperature (°C), humidity (%) and cloud cover (as a %) were recorded.

For the first three habitats the transect layout described above and shown in figure 2.3 was used. However, for the last three habitats the difficult terrain meant that VES were carried out along established transects for 500 metres. A summary of the transects and their relative distance from one another can be seen in figure 2.2.
Figure 2.3: Diagram showing the transect layout used for the first three habitats, highlighting the length of each transect and the direction of walking.

**Stream quadrats**

Streams which flow through Kerangas forests are tea-coloured due to the presence of organic colloids, are acidic (pH 3-4.5) and have low oxygen content due to lack of aquatic vegetation, low light penetration and few inorganic ions (MacKinnon et al., 1996). The productivity of these streams is very low and many of the fish found within them are facultative air breathers (MacKinnon et al., 1996). The various streams which ran throughout the habitat were surveyed using twenty 10x10 metre plots set-up and surveyed over seven days. The location of these quadrats is shown in figure 2.2. These were split up into ten fast flowing streams and ten slow flowing streams. The plots were marked out
during the day using brightly coloured raffia and each night either two or three quadrats were sampled. Two persons, one experienced personnel and a local guide, surveyed each plot by starting at opposite corners and as silently as possible searching the entire area for amphibians. The same protocol as VES was used for each individual encountered.

**Other methods**

Four pitfall and four funnel traps, separated by drift fences, were placed in the kerangas and mixed peat habitats for ten nights each, but failed to produce useful results. These were discontinued and the results not included. The quadrat method was also used by setting up $10m^2$ plots during the day in the kerangas habitat; however this method failed to find any individuals so was also discontinued. Calls heard either on transects or opportunistically were included as data points. Calls which could not be identified were noted (time and place) and recorded using a Dictaphone on later VES. Once out of the field the recordings were sent to experts to confirm their identities. Two tadpoles which were encountered on the second day were caught using a hand net and transferred to a plastic tank. They were kept for 52 days, until the end of the time in the field, being fed on dead leaves. Photographs were taken twice to show the progress of their development. Once out of the field the photographs were sent to various experts to help with the identification.
Environmental variables

Temperature (°C), humidity (%) and cloud cover (as a %) were measured at the start of each day and night transects and stream quadrat. The temperature and humidity were recorded on a thermo-hygrometer (from alanaecology.com) and the cloud cover by eye. At camp the minimum and maximum temperatures of the day were recorded on a minimum and maximum thermometer, though the data were deemed unreliable and were not included in the data analysis. The rainfall was collected in a rain gauge and measurements taken twice daily at 7am and 7pm.

MacKinnon’s List

This method is typically used for avifaunal surveys with the lists containing ten or 20 species (O’Dea et al., 2004). However, as the number of species of amphibians is much lower than the number of birds, instead each list contained five species. The time at which every individual was encountered, either during VES, quadrat or opportunistically, was recorded to allow species to be organised into lists. Each list is made up of five different species in the order encountered, with no list containing the same species twice. If two or more individuals of the same species are encountered whilst making a list, only the first individual would be included.
**Statistical analysis**

The Shannon Index is a measure of species diversity. Species diversity takes into account the relative abundance and evenness of species as well as the overall number of species in an area. Therefore species diversity gives a more complete value than species richness alone. The Shannon Indices were calculated on Diversity package for each night VES were carried out. The results were then compared between habitats using a General Linear Model and a Tukey Test on Minitab.

To compare between habitats the data for each method were uploaded to EstimateS programme, ‘a free software application that computes a variety of biodiversity functions, estimators, and indices based on biotic sampling data’ (Colwell, 2009). Several species richness estimators were computed including ACE (Abundance-based Coverage Estimator) and Chao1, which are for abundance-based data, and Chao2 and ICE (Incidence-based Coverage Estimator) which are for sample-based data (Colwell, 2009). These estimates were compared between the two methods to see if different total species numbers are produced. The output also includes Sample-based Rarefaction which is used to produce species accumulation curves for each method in excel. From eye it can be judged whether the curves are reaching an asymptote or not and therefore allows a conclusion on whether the site has been adequately surveyed or not.

Individuals were not marked (e.g. toe clipping), therefore only relative abundance could be calculated. For VES this was done by dividing the total number of individuals of one species by the total number of individuals. For MacKinnon’s List the number of lists a
species occurred in was divided by the total number of lists. The data were then ranked so a top ten was worked out for each method.

**Conservation status**

Each species was entered into the IUCN Red List 2010 (IUCN, 2010) to determine their IUCN status and population trend.

![Nyctixalus pictus](image)

**Figure 2.4:** *Nyctixalus pictus* - only one individual found in Kerangas plus a stream habitat. Listed as Near Threatened by IUCN (Picture by Iván Mohedano)
**Results**

**Sampling effort**

Throughout the surveying period 421 individuals were encountered comprising of 18 (possibly 19) different species, all anurans. No caecilians or salamanders were encountered. A species inventory including all species caught (VES and opportunistically) is shown in figure 3.1. It was decided to only include data from the first five habitats (kerangas, mixed-peat, kerangas with a stream, burnt new and burnt regenerating) in the analysis. The calls, tadpoles and individuals caught in traps and on day transects were excluded as several calls and the tadpoles could not be identified and because the traps and day transects failed to produce useful results (four individuals over 34 days and four individuals over 31 days respectively). These individuals were species that had been already encountered on nocturnal VES. Data for analysis were collected over a period of 35 days, totalling 259.17 nocturnal person hours. A summary of the surveying effort is shown in figure 3.2 and includes the days spent on each method and the number of lists and person hours per habitat.

**Species Inventory**

The complete species list is shown in figure 3.1. It shows which species were found in which habitat (X) and also includes the percentage of each species caught by VES for each habitat in brackets. Those which only have an X and no bracket were opportunistic encounters. C = camp, K= kerangas, M-PS = mixed-peat swamp, K+S = kerangas plus
stream, BN = burnt new, BR = burnt regenerating and LP = low interior forest. A rough value of species richness is shown in the number of species encountered per habitat in the total row of the table.

<table>
<thead>
<tr>
<th>Species</th>
<th>C</th>
<th>K</th>
<th>M-PS</th>
<th>K+S</th>
<th>BN</th>
<th>BR</th>
<th>LP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bufonidae</td>
<td></td>
<td>X</td>
<td></td>
<td></td>
<td>X (2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fejervarya cancrivora</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>X (13)</td>
<td></td>
</tr>
<tr>
<td>Hylarana baramica</td>
<td>X</td>
<td>X (6)</td>
<td>X (22)</td>
<td>X (12)</td>
<td>X (4)</td>
<td>X (33)</td>
<td>X (67)</td>
</tr>
<tr>
<td>Hylarana glandulosa</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hylarana raniceps</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>X (2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ignerophrynus quadriforcat</td>
<td>X</td>
<td>X</td>
<td>X (2)</td>
<td>X (12)</td>
<td>X (2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kalophrynus sp.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kalophrynus pleurostigma</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>X (7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leptobrachium spp.</td>
<td></td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Limnonectes malesianus</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>X (9)</td>
<td></td>
</tr>
<tr>
<td>Limnonectes paramacrodon</td>
<td>X</td>
<td></td>
<td></td>
<td>X (23)</td>
<td>X (2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Microhyla borneensis</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nyctixalus pictus</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>X (2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Occidozyga laevis</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Polypedates colletti</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>X (2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Polypedates leucomystax</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td>X (4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Polypedates macrotis</td>
<td>X</td>
<td>X (8)</td>
<td>X (4)</td>
<td>X (9)</td>
<td>X (90)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rhacophorus appendiculatus</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>6</td>
<td>7 (5)</td>
<td>11 (11)</td>
<td>10 (10)</td>
<td>6 (5)</td>
<td>6 (5)</td>
<td>2 (2)</td>
</tr>
</tbody>
</table>

**Figure 3.1:** The complete list of species found in each during the surveying period, including the percentages of individuals caught by VES method in brackets. The total numbers of species, for each habitat, are included in the bottom row with the number caught by VES alone in brackets. Those species which are only found in one habitat are highlighted in red.

Figure 3.1. shows that mixed-peat swamp has the highest number of species caught in total and by VES, followed by kerangas with stream, kerangas, burnt new, burnt regenerating and low pole, which has the lowest number of species encountered. At camp six species were opportunistically encountered, this site would be classed as a forest edge habitat. Bufonidae and Kalophrynus sp. were only encountered once in mixed-peat swamp and could not be identified to species level, Bufonidae only to family.
*Leptobrachium spp.* was encountered several in different habitats but it could not be identified to species level. Either one or two species may have been present.

**Surveying effort**

Figure 3.2 is a table summarising the survey effort per habitat for each method.

<table>
<thead>
<tr>
<th>Habitat type</th>
<th>MacKinnon’s List</th>
<th>Visual Encounter Survey</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Days of collection</td>
<td>Number of Lists</td>
</tr>
<tr>
<td>Kerangas</td>
<td>10</td>
<td>1</td>
</tr>
<tr>
<td>Mixed-peat swamp</td>
<td>10</td>
<td>6</td>
</tr>
<tr>
<td>Kerangas+stream</td>
<td>7</td>
<td>6</td>
</tr>
<tr>
<td>Burnt new</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>Burnt regenerating</td>
<td>4</td>
<td>1</td>
</tr>
</tbody>
</table>

**Figure 3.2**: Survey effort for the five habitat types for MacKinnon’s List and VES, showing the days spent collecting the data and the numbers of lists or person hours compiled. Lists compiled per habitat for the same 35 days VES were carried out.

**Environmental variables**

The environmental variables recorded during the survey were not included in the analysis. Both temperature and humidity were recorded at the start of each of the 31 VES and the averages and standard deviations of both calculated and shown in figure 3.3. The averages of the entire survey period could not be calculated as the maximum and minimum thermometer at camp broke and there was no permanent hygrometer. The rainfall was recorded twice a day for the entire survey period which totaled 54 days.
Figure 3.3: Summary of environmental variables recorded. Temperature and humidity recorded at the start of VES and rainfall recorded throughout the entire period in the field. (total rainfall – am and pm refers to time the recording was taken not rainfall period, so am records rainfall overnight and pm records rainfall throughout the day).

Temperature has a very low standard deviation which shows there is little variation between each night. Humidity has a higher standard deviation which shows there is slightly more variation between nights. Rainfall am average is much higher then rainfall pm average showing a distinct difference in the volume of rain between night and day.

The standard deviation for am is much higher than pm which shows there is a lot of variation between nights. Either it rained all night or not at all but during the day it usually rained around the same time (late afternoon) for the same period (one hour).

**Species Diversity**

Shannon Indices were calculated for each night at each site using Diversity package (Pisces Conservation Ltd, 2011). The results are shown below in figure 3.4.
Figure 3.4: Boxplot showing the range of Shannon indices for each site. A-kerangas, B-mixed-peat, C-kerangas+stream, D-burnt new and E-burnt regenerating. The Shannon index was calculated for each individual night at each site. Ten nights were spent in sites A & B, seven nights in site C and four nights in sites D and E. The centre line in each plot refers to the median of the sample, the vertical line refers to the range, the upper and lower lines of the box refer to the upper and lower quartiles of the sample respectively. The asterisk refers to an outlier data point. The mean values are displayed adjacent to each box.

The box plot implies there is a difference in species diversity between the five sites. To confirm if there is a significant statistical difference and to reject the null hypothesis, that there is no difference between the five sites, a General Linear Model was carried out. A GLM was made on Minitab and the output shown below in figure 3.5.

General Linear Model: H versus Site

<table>
<thead>
<tr>
<th>Factor</th>
<th>Type</th>
<th>Levels</th>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Site</td>
<td>fixed</td>
<td>5</td>
<td>A, B, C, D, E</td>
</tr>
</tbody>
</table>
Analysis of Variance for \( H \), using Adjusted SS for Tests

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>Seq SS</th>
<th>Adj SS</th>
<th>Adj MS</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Site</td>
<td>4</td>
<td>3.5900</td>
<td>3.5900</td>
<td>0.8975</td>
<td>5.89</td>
<td>0.001</td>
</tr>
<tr>
<td>Error</td>
<td>30</td>
<td>4.5715</td>
<td>4.5715</td>
<td>0.1524</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>34</td>
<td>8.1615</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\( S = 0.390362 \)  \( R-Sq = 43.99\% \)  \( R-Sq(adj) = 36.52\% \)

Unusual Observations for \( H \)

<table>
<thead>
<tr>
<th>Obs</th>
<th>H</th>
<th>Fit</th>
<th>SE Fit</th>
<th>Residual</th>
<th>St Resid</th>
</tr>
</thead>
<tbody>
<tr>
<td>11</td>
<td>1.73300</td>
<td>0.91896</td>
<td>0.12344</td>
<td>0.81404</td>
<td>2.20 R</td>
</tr>
<tr>
<td>12</td>
<td>0.00000</td>
<td>0.91896</td>
<td>0.12344</td>
<td>-0.91896</td>
<td>-2.48 R</td>
</tr>
<tr>
<td>16</td>
<td>0.00000</td>
<td>0.91896</td>
<td>0.12344</td>
<td>-0.91896</td>
<td>-2.48 R</td>
</tr>
</tbody>
</table>

\( R \) denotes an observation with a large standardized residual.

**Figure 3.5:** GLM output for Species Diversity (\( H \)) versus Site (A,B,C,D,E). Significant \( p \)-values are highlighted in red.

The GLM gives a \( p \)-value of 0.001 which means the null hypothesis can be rejected as there is a significant difference in diversity between the habitats. The R-Sq value is 43.99\% which means just under 44\% of the variation in diversity is explained by the different habitats. To calculate which sites are significantly different from one another a Tukey test was carried out and the results shown in figure 3.6.

**Tukey Simultaneous Tests**

Response Variable \( H \)

All Pairwise Comparisons among Levels of Site

Site = A   subtracted from:

<table>
<thead>
<tr>
<th>Site of Means</th>
<th>SE of Difference</th>
<th>T-Value</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>B</td>
<td>0.40175</td>
<td>0.1746</td>
<td>2.3013</td>
</tr>
<tr>
<td>C</td>
<td>0.81255</td>
<td>0.1924</td>
<td>4.2238</td>
</tr>
<tr>
<td>D</td>
<td>-0.09339</td>
<td>0.2309</td>
<td>-0.4044</td>
</tr>
<tr>
<td>E</td>
<td>0.49154</td>
<td>0.2309</td>
<td>2.1284</td>
</tr>
</tbody>
</table>

Site = B   subtracted from:

<table>
<thead>
<tr>
<th>Site of Means</th>
<th>SE of Difference</th>
<th>T-Value</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>0.41075</td>
<td>0.1924</td>
<td>2.3013</td>
</tr>
<tr>
<td>D</td>
<td>-0.4951</td>
<td>0.2309</td>
<td>-2.144</td>
</tr>
<tr>
<td>E</td>
<td>0.0898</td>
<td>0.2309</td>
<td>0.389</td>
</tr>
</tbody>
</table>

Site = C   subtracted from:
<table>
<thead>
<tr>
<th>Site</th>
<th>Difference</th>
<th>SE of Difference</th>
<th>T-Value</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>D</td>
<td>-0.9059</td>
<td>0.2447</td>
<td>-3.703</td>
<td>0.0071</td>
</tr>
<tr>
<td>E</td>
<td>-0.3210</td>
<td>0.2447</td>
<td>-1.312</td>
<td>0.6860</td>
</tr>
</tbody>
</table>

Site = D subtracted from:

<table>
<thead>
<tr>
<th>Site</th>
<th>Difference</th>
<th>SE of Difference</th>
<th>T-Value</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>E</td>
<td>0.5849</td>
<td>0.2760</td>
<td>2.119</td>
<td>0.2386</td>
</tr>
</tbody>
</table>

**Figure 3.6:** Output of GLM showing Tukey test results. Significant p-values are highlighted in red.

The results of the Tukey test show that diversity indices for sites A and C were significantly different from one another as were sites C and D. The rest had p-values of >0.05 which shows no significant difference in diversity between those sites.

**Species accumulation curves**

Species accumulation curves for both VES and MacKinnon’s Lists were plotted using EstimateS program (Colwell, 2009), with sample number (transect night and list number respectively) against number of species discovered. This is shown in figure 3.7.
Figure 3.7: Species accumulation curves for VES (blue) and MacKinnon’s List (pink) method. The lists are made up regardless of habitat for the 35 sampling days VES were carried out.

The curves shown in figure 3.7 have not reached an asymptote which suggests Bawan forest was not adequately surveyed by both methods. The curves are almost equal although the MacKinnon’s List curve appears to be reaching an asymptote before the VES curve.

Species Richness Estimation

Different species richness estimators were calculated using EstimateS program (Colwell, 2009) for both methods. The results can be seen below in figure 3.8.

Figure 3.8: Graph showing the different species richness estimations for different methods.

Figure 3.8 shows the computed species estimations by EstimateS (Colwell, 2009) for each method. Species richness estimators include ACE (Abundance-based Coverage
Estimator) and Chao1, which are for abundance-based data, and Chao2 and ICE (Incidence-based Coverage Estimator) which are for sample-based data (Colwell, 2009). Overall the estimators produce similar results for both methods, the lowest being 19.44 and the highest being 23.49. MacKinnon’s List method calculates slightly lower species estimations than VES for each estimator. For both Chao estimators 95% confidence intervals were computed, shown in figure 3.9. MacKinnon’s List method showed a much narrow range for both Chao1 and Chao2 than VES.

<table>
<thead>
<tr>
<th>Chao1 CI Lower</th>
<th>Chao1 CI Upper</th>
<th>Chao2 CI Lower</th>
<th>Chao2 CI Upper</th>
</tr>
</thead>
<tbody>
<tr>
<td>VES 16.78</td>
<td>48.14</td>
<td>17.28</td>
<td>57.4</td>
</tr>
<tr>
<td>Mac 18.18</td>
<td>30.47</td>
<td>18.17</td>
<td>30.12</td>
</tr>
</tbody>
</table>

**Figure 3.9:** Table showing the lower and upper values for the 95% confidence interval for Chao1 and Chao2 as computed by EstimateS (Colwell, 2009).

**Relative abundance**

The relative abundance was calculated from the data collected in the five habitats for VES and MacKinnon’s List and the results shown in figure 3.10. The MacKinnon’s Lists were made up of all individuals collected in these habitats both from VES and opportunistically.

<table>
<thead>
<tr>
<th>Species name</th>
<th>VES</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>MacKinnon’s List</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
### Figure 3.10: Table showing where each species ranks in the top ten of relative abundance of both VES and MacKinnon’s List methods. MacKinnon’s List was calculated in three different ways. 1 – lists made up for each habitat for the entire period in the field (54 days), 2 – lists made up for each habitat for the 35 VES sampling days and 3 – lists made up regardless of habitat for the 35 VES sampling days.

The top ten most abundant species for both VES and all MacKinnon’s List have a 90% crossover with only one species occurring in VES and not the in lists. The rank order is different in all four but the upper and lower five (separated by the red line) are the same. The three different forms of MacKinnon’s List were compiled to show the different results produced.

<table>
<thead>
<tr>
<th>Species</th>
<th>VES Rank</th>
<th>MacKinnon’s List 1</th>
<th>MacKinnon’s List 2</th>
<th>MacKinnon’s List 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polypedates colletti</td>
<td>1</td>
<td>=3</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>Polypedates macrotis</td>
<td>2</td>
<td>5</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>Hylarana baramica</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Occidozyga laevis</td>
<td>4</td>
<td>2</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Limnonectes paramacrodon</td>
<td>5</td>
<td>=3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Leptobrachium spp.</td>
<td>6</td>
<td>6</td>
<td>8</td>
<td>6</td>
</tr>
<tr>
<td>Hylarana raniceps</td>
<td>7</td>
<td>=7</td>
<td>=6</td>
<td>=7</td>
</tr>
<tr>
<td>Kalophrynus pleurostigma</td>
<td>8</td>
<td>9</td>
<td>9</td>
<td>=9</td>
</tr>
<tr>
<td>Limnonectes malesianus</td>
<td>9</td>
<td>=7</td>
<td>=6</td>
<td>=9</td>
</tr>
<tr>
<td>Fejervarya cancrivora</td>
<td>10</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ignerophrynus quadriporocatus</td>
<td>10</td>
<td></td>
<td></td>
<td>=7</td>
</tr>
</tbody>
</table>

The top ten most abundant species for both VES and all MacKinnon’s List have a 90% crossover with only one species occurring in VES and not the in lists. The rank order is different in all four but the upper and lower five (separated by the red line) are the same. The three different forms of MacKinnon’s List were compiled to show the different results produced.
**Tadpoles and foam nests**

The two tadpoles which were caught and grown back at camp could not be formally identified though experts confirmed them to belong to the genus *Leptobrachium*, based on morphology and habitat. One other froglet encountered by VES (Kerangas+stream) was believed to also belong to this genus.

The foam nests discovered on the kerangas plus stream transects were photographed (figure 3.11) along with the tadpoles found in the pool under the nests and sent to experts for identification. They too could not be identified down to species level but were confirmed to belong to the genus of *Rhacophorus*.

![Foam nest found in third habitat, kerangas with stream (Picture by Iván Mohedano)](image-url)
Breeding pond and incidental observations

In the fourth habitat (burnt new) there were two pools as described in the methods. During the surveying period in this habitat these were used as breeding ponds for the treefrog *Polypedates macrotis*. In one night survey 25 individuals were encountered, mostly calling, around one pool. On one occasion a male and a female were seen in amplexus, shown in figure 3.12. Calling males of the tree frog *Rhacophorus appendiculatus* were also recorded by this pool.

On one day transect in the third habitat (kerangas + stream) a frog was located in a stream after hearing it scream. It was discovered being eaten by the water snake *Xenochrophys trianguligerus* (red-sided keelback).

Figure 3.12: Male and female *Polypedates macrotis* in amplexus in the burnt new habitat (Picture by Joanna Klys)
Discussion

Species diversity

Figure 3.4 shows that on average kerangas with stream was the most species diverse habitat and the least was burnt new. Burnt regenerating was the second, followed closely by mixed-peat swamp and then by kerangas. These results were confirmed by the subsequent GLM and Tukey test shown in figures 3.5 and 3.6, which show statistically significant differences between kerangas with stream and both burnt new and kerangas. As shown in figure 3.1, based on number of species alone mixed-peat swamp forest was the most species rich habitat with eleven in total. However this habitat only ranked as the third most diverse and its large variation throughout the surveying period is displayed in figure 3.4. Two out of the ten night surveys had to be cut short due to medical reasons and bad weather; during the latter no individuals were found which lower the overall species diversity for this habitat. The torrential rain which forced the survey to stop will most likely have not affected the numbers of individuals in the area but instead human error will have resulted in no individuals being encountered. It appears that the species diversity has not been affected by length of study as both burnt new and burnt regenerating were only surveyed for four days, with the former being the least diverse and the latter ranking second. Kerangas with stream was surveyed for seven days, three days less than both mixed-peat swamp and kerangas, though was ranked as having the highest diversity. Muir (in press) came to the conclusion that ‘MacKinnon’s List data are suboptimal for the calculation of diversity indices’, further backed by observations in
O’Dea et al., (2004) and Poulsen et al., (1997) which is why only VES data were included in species diversity indices.

Kerangas with stream was the most diverse habitat being significantly different (p-value = 0.0018) from kerangas alone, despite both being heath forest. Kerangas with stream was a much wetter habitat with a stream cutting through each of the five transects at least once, as well as several permanent and ephemeral pools. Due to their amphibious life history this habitat would be ideal for populations of anurans and evidence for this is that it was the only site which had tadpoles and foam nests. These tadpoles were only identified down to genus level and adult individuals of the genus *Rhacophoridae* to which they belong, were not encountered in this habitat. This genus includes tree frogs that have several species living in the canopy coming down only to breed (Inger and Stuebing, 2005). VES as a technique is biased towards fossorial and semi-arboreal species, e.g. *Hylarana* and *Leptobrachium*. A large number of tropical species are arboreal (Kays and Allison, 2001) making it very likely that a different arboreal species not encountered lives in this habitat and produced these nests. Several calls were heard from the canopy whilst on transect, although could not be identified therefore it cannot be confirmed whether they were from a new species or not. The forest in kerangas with stream was more varied than kerangas alone with a lot more large dipterocarp trees, a higher canopy and emergents. The area had been selectively logged with old felled trees on transect which opened up the forest and canopy. The forest edge was met at one end of each transect (except the fifth) which provided a new habitat for generalist forest edge species. Kerangas habitat was in the centre of Bawan forest and was more uniform in
structure with lots of small trees, which may explain its low diversity. The majority of species encountered occupied primary and old secondary peat forests.

As Shannon Index takes into account relative abundance the low diversity in Kerangas may be due to the very high numbers of *Polypedates colletti* encountered (72% of individuals). Kerangas with stream had more even percentages with the highest being just 27%. Unexpectedly only one individual of *Polypedates colletti* was encountered in kerangas with stream despite its large numbers in kerangas and it being the most numerous species caught by VES. This observation cannot be explained by previous studies as there is limited literature on the ecology of this species. Aggregations of individuals do occur around temporary rain pools during breeding (IUCN, 2010). However no such pools were observed during the survey in kerangas habitat. Another possibility is that the forest was denser, with smaller closer trees, which might be a more suitable habitat for the tree frog compared with the more open kerangas with stream.

The burnt new habitat was statistically the least diverse whereas the similar habitat, burnt regenerating, had the second highest diversity. As mentioned above Shannon Index takes into account relative abundance and although five species were encountered in both habitats the presence of large numbers (90%) of one species encountered in burnt new will have affected the diversity. The dominance of *Polypedates macrotis* in the burnt new habitat is likely to have skewed diversity towards a less diverse figure. These individuals were encountered perching on submerged branches around two permanent pools, most were male and were calling. Such behaviour had not been observed anywhere or at any
other time in Bawan forest. Despite the lack of trees these tree frogs may have chosen to breed here because these species may favour the alkaline water of the charcoal pool compared to the highly acidic peat water of the streams and pools. However, there is no published literature on the breeding preferences of this species so this cannot be confirmed. Another possible reason is due to the presence of the full moon on the third survey night (out of four). Previous studies have shown evidence that the moon phase affects breeding in some European anurans, with higher incidences at a full moon (Grant et al., 2009), though this has not been previously observed in this species. A different species of tree frog (*Rhacophorus appendiculatus*) that not encountered in any other habitat was also observed in burnt new habitat, calling from shrubs next to the same pool. Only one individual was encountered by VES though four more were found opportunistically and were therefore included in MacKinnon’s Lists. As previously mentioned it is an unusual site for tree frogs as the habitat very open with few trees and shrubs making individuals more susceptible to predation from nocturnal birds. It is surprising that this species was only found in this habitat particularly as its call made it so conspicuous; each individual encountered was heard first. One explanation may be this species does occur in the other habitats and these individuals were there only for breeding, like *P. macrotis*, because of this particular pool, the moon phase or other reason. *Rhacophorus appendiculatus* is reported to inhabit lowland swampy forests and forest edge habitats, breeding in stagnant waters (Haas, 2010). Although the results suggest this habitat is low in overall diversity, the particular species which were found and their abundance makes this a very interesting site for amphibians, especially when considering the impacts of habitat disturbance on amphibian populations. However an important point
to remember is that this disturbed area is only 5000 m\(^2\) in size and is completely surrounded by primary forest. A larger area not in contact with undisturbed forest would most likely not support the same level of diversity.

The burnt regenerating habitat was difficult to survey as the vegetation was impenetrable therefore only the area of road was searched. Several calls could be heard from the shrubs though the individuals could not be located. Recordings of these individuals were made though could not be identified by experts, so it is unknown whether these were from different species or not. The presence of *Hylarana baramica* and *Leptobrachium spp.* were not unusual as several individuals were seen around camp. *Occidozyga laevis* was the most numerous species found and was always found in puddles in the sand (formed by tyre marks), the usual habitat for this species. The mangrove frog (*Fejervarya cancrivora*) was encountered on the last survey night and then opportunistically when sound recording. Three individuals were spotted on both nights but only one was caught and photographed. Despite being a large, stocky frog (Inger and Stuebing, 2005) individuals hid very well in the puddles and when disturbed moved rapidly into the surrounding vegetation. This explains why more individuals were not encountered. Although, as the name suggests, this frog is usually restricted to coastal regions, populations do occur inland in Borneo (Frost, 2010). This species tends to inhabit disturbed habitats preferring ditches and river banks (Inger and Stuebing, 2005) which explains why it was only found in this habitat. The road leads to Bawan village where the population may have originated before spreading east into the burnt forest. As the village is 10km away individuals may have been accidentally exported by vehicles, rather than
moving this distance naturally. Two individuals of *Kalophrynus pleurostigma* were encountered in this habitat. This species is a forest specialist occurring only in lowland primary forest (Inger and Stuebing, 2005). As the transect was not far from the forest edge it is possible that some adult individuals may have moved into this habitat. Without conducting a long term study in the area it is impossible to draw any conclusions about the future of populations in this habitat. If populations were to thrive over time this would be very interesting for conservation and the regeneration of lowland rainforest. It is unusual that the species *Polypedates leucomystax* and *Hylarana glandulosa* were not found in this habitat as both were encountered opportunistically at camp. These species may still be present in this habitat but were just not encountered due to the dense vegetation.

Mixed-peat swamp habitat has the highest overall species number despite being the smallest in area out of the six in Bawan forest. As shown in figure 2.2, only a couple of 100 metres along the TA transect was mixed-peat swamp. However, the transition between the different habitats was distinct with indicator plant species confirming habitats. As previously mentioned, the highest number of species (11) were encountered in this habitat, 61% of species found in Bawan forest. Included in this list were two species that only one individual were encountered, *Kalophyrrnus sp.* and *Bufonidae* species. As these could potentially be new species this small habitat is extremely important for amphibian diversity.

**VES versus MacKinnon’s List method**
MacKinnon’s List method produced a higher number of species than VES method (18 compared with 16), shown in figure 3.7, suggesting that it is a more useful technique to discover species richness. This method encountered two more species as it included opportunistic encounters. *Hylarana grandulosa* and *Polypedates leucomystax* were found only around camp and could therefore not be included by VES as they were never found on a transect. The species accumulation curves shown in figure 3.7 conclude that neither method adequately surveyed the area in the available time. Although both are reaching an asymptote, MacKinnon’s List method earlier than VES, species were still being discovered towards the end of survey period suggesting there are probably more species present in the area. The curves also show that MacKinnon’s List method continually recorded higher species richness throughout the survey period. Species richness estimators were computed to estimate the overall number of species inhabiting Bawan forest, including those which were not encountered during the survey. As displayed in figure 3.8 the estimators all produced similar values for both methods, with the lowest being 19.44 and the highest being 23.49. It is very likely that the true species number does lie between these figures. For both Chao estimators lower and upper confidence intervals were computed and for both, MacKinnon’s List method had a much narrower range, suggesting the mean values are more accurate.

Figure 3.10 shows that all four relative abundance lists were very similar with a 90% crossover. The top five and the lower five for each method contain almost the same species (apart from *F. cancrivora* only appearing in VES and being replaced by *I. quadriporcutus* for MacKinnon’s List). This suggests very similar results have been
produced for both methods. The rank order for VES shows the most numerically abundant species by VES in Bawan forest. *Polypedates colletti* was number one as it appeared in high numbers in both kerangas and mixed-peat habitats, the two habitats that were surveyed for the longest period (ten days each). *Hylarana baramica* was ranked first for all MacKinnon’s Lists being a frequent opportunistically encountered species, as groups lived next to camp and were seen when leaving for and returning from VES. Although this species was not the highest numerically, it was found in every habitat, the only species to do so. *Polypedates macrotis* is the second most abundant species according to VES and MacKinnon’s List three, though fifth for MacKinnon’s Lists one and two. It was ranked second for VES due to the large number seen breeding in the burnt new habitat. As species are only included once in a list, regardless of the number encountered, 25 individuals were only included as one encounter appearing in one list, which is why it has ranked lower for MacKinnon’s Lists one and two. This has been noted as a bias in MacKinnon’s List method, underestimating flocking species in avian surveys (O’Dea et al., 2004). This is the first bias detected with MacKinnon’s List method which suggests that relative abundance calculations are unreliable. A second bias is the order in which species are recorded affects their abundance. If two individuals of the same species were encountered half way through a list only one would be included. If they were encountered at the end both will be included, as the last species in the first list and as the first in the second list, doubling their abundance. The three different MacKinnon’s List abundance lists were compiled in order to show how arranging the data in slightly different ways can change the overall relative abundance. However it was concluded that the best list out of the three was the third – lists made up regardless of
habitat for the 35 VES sampling days. Although VES method was separated into different habitats, the data was compiled in one Bawan forest data set for comparison with MacKinnon’s List data. Therefore the data for VES and all opportunistic encounters (regardless of habitat) for the same 35 days should be compiled (in chronological order) and the lists made accordingly for a fair comparison. This was the list that was used for the MacKinnon’s List accumulation curves and species estimations, in figure 3.7 and 3.8 respectively.

MacKinnon’s List method takes into account opportunistic encounters therefore does not waste data and uses time more efficiently. In these respects it is better than VES method. It produced slightly more accurate species richness estimations and appeared to be reaching an asymptote first but overall these were very similar results for the two methods so one cannot be concluded to be better. As previously mentioned a large number of tropical anurans are arboreal, rarely spotted apart from when coming down to breed. MacKinnon’s List method allows these species to be included in lists by including their calls as data points. This would work very well in areas where the arboreal anuran calls were well known and can be studied from recorded CDs beforehand, e.g. in the Amazon. Unfortunately such information is not available for these species in Borneo. There are no CDs available, only a dozen recordings on www.frogsofborneo.org (four of which are arboreal) and a vague description in Inger and Stuebing (2005). More canopy surveys need to be carried out with emphasise on sound recording to allow the calls of these species to be identified and included in future inventories.
Species mis-identification and potential for new species

Amphibians are known to be extremely variable as so many different morphs of the same species exist (e.g. Polypedates colletti) as well as geographical variation (Barlett and Barlett, 2003). Some of the frogs that were unable to be identified may have been different morphs of the same species or potential new species, for example Leptobrachium spp. and Kalophyrnus sp. One way to solve this problem would be to introduce DNA profiling of tissue samples, to conclude whether or not a particular individual belongs to a specific described species or if it is new to science. However, this technique will increase the costs of the study greatly and the exportation of animal products can be very difficult in some countries. A few species caught during the survey could not be identified in the field, and pictures had to be sent to various experts to confirm identities. Even then some were disputed and a few could only be identified down to genus level and one down to family level. This could mean that some of the individuals encountered may turn out to be new species to science, but without voucher specimens this cannot be confirmed. OuTrop as an organisation are heavily focused on conservation of all wildlife and as only one individual was encountered for two of the unidentified species it would have been unjustified to take voucher specimens. In the future OuTrop should consider taking tissue samples to confirm unknown species. Knowing what species occur and where is particularly important in south-east Asia as there is a lot of scope for finding new species, especially in Indonesian Borneo where little herpetological research has been carried out (IUCN, 2008). The IUCN predict Indonesia to be the most amphibian rich country outside the Americas and that probably only half of the species are known yet (IUCN, 2008).
Limitations of this study

- Length of time spent in each habitat should have been equal. Bad weather meant that time spent in the third habitat (kerangas with stream) had to be shortened from ten to seven days and time constraints meant that only four days could be spent in the fourth habitat (burnt new).

- A more extensive survey of the low interior forest would have been interesting, though would have involved camping and was not feasible for this study. A camping trip to record gibbons at 2.5km along TA (see figure 2.2) observed many frogs, most of which were calling.

- Environmental variables such as altitude, moisture, rainfall, soil moisture, soil temperature, air temperature and humidity are known to affect amphibian richness (Heyer, 1994 and Pearman, 1997). It would have been worthwhile to look at whether environmental data affected the distribution of species in different habitat in Bawan forest, as differences have known to affect amphibian species diversity on a local scale (Laurencio and Fitzgerald, 2010). A longer study over a year could have looked at seasonal fluctuations in environmental variables.

- Although great care was taken in identifying every individuals mis-identification of species may have occurred which obviously would have affected diversity.

- Some species may have been underestimated due to sampling limitations as VES only really samples fossorial and semi-arboreal species. Four species were only represented by one individual though many more may have been present and just not encountered. Microhyla borneensis, only caught once by VES, is extremely
small and inconspicuous and is hard to catch due to its disproportionately large hind limbs (Inger and Stuebing, 2005). VES alone may have underestimated the number of individuals of this species.

- The lack of knowledge of species calls in Borneo meant that even those that were recorded could not be identified by call alone. This created a bias towards arboreal species that could be heard but not seen, as they were not included in the data set unlike semi-arboreal and fossorial species that could be located after hearing their call.

- Anurans found in the canopy (which may have been calling) were not surveyed. Canopy surveys are known to improve species richness assessments and inventory efficiency in tropical rainforests (Kalko and Handley, 2001; Longino and Colwel, 1997). VES are biased against those individuals found in the canopy (Heyer et al., 1994). Kays and Allison (2001) highlighted the need for more amphibian canopy surveys in the tropics.

- More than one transect should have been surveyed each night in order to give a better representation of the community and to use the time more efficiently.

- Inexperience of the samplers at the beginning and the changing of volunteers and guides each night may have effect the numbers encountered as some were better than others at spotting and catching individuals.

- Overall abundance could not be looked at as the individuals were not marked. It was decided not to use toe-clipping to mark individuals as adverse effects have been observed in previous studies (May, 2004).
Niche preferences could not be looked at due to lack of data. The exact location on the transects where individuals were caught could be noted in order to look at preferences of each species. This may also determine if individuals are being recaptured.

**Conservation implications**

Figure 4.1 displays the most up-to-date IUCN status and population trend of all species found in Bawan forest. Those in a higher risk category than least concern and with a decreasing trend are highlighted in red.

<table>
<thead>
<tr>
<th>Species</th>
<th>Family</th>
<th>IUCN status</th>
<th>Population trend</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Bufo</em> sp.</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td><em>Fejervarya cancrivora</em></td>
<td>DICROGLOSSIDAE</td>
<td>Least Concern</td>
<td>Increasing</td>
</tr>
<tr>
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<td>Least Concern</td>
<td>Decreasing</td>
</tr>
<tr>
<td><em>Hylarana glandulosa</em></td>
<td>RANIDAE</td>
<td>Least Concern</td>
<td>Decreasing</td>
</tr>
<tr>
<td><em>Hylarana raniceps</em></td>
<td>RANIDAE</td>
<td>Least Concern</td>
<td>Stable</td>
</tr>
<tr>
<td><em>Ingerophrynus quadriporcatus</em></td>
<td>BUFONIDAE</td>
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<td>Unknown</td>
</tr>
<tr>
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<td>Least Concern</td>
<td>Stable</td>
</tr>
<tr>
<td><em>Kalophrynus spp.</em></td>
<td>MICROHYLIDAE</td>
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<td>N/A</td>
</tr>
<tr>
<td><em>Leptobrachium spp.</em></td>
<td>MEGOPHYRIDAE</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td><em>Limnonectes malesianus</em></td>
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<td>Near threatened</td>
<td>Decreasing</td>
</tr>
<tr>
<td><em>Limnonectes paramacrodon</em></td>
<td>DICROGLOSSIDAE</td>
<td>Near threatened</td>
<td>Decreasing</td>
</tr>
<tr>
<td><em>Microhyla borneensis</em></td>
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<td>Least Concern</td>
<td>Decreasing</td>
</tr>
<tr>
<td><em>Nyctixalus pictus</em></td>
<td>RHACOPHORIDAE</td>
<td>Near threatened</td>
<td>Decreasing</td>
</tr>
<tr>
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<td>DICROGLOSSIDAE</td>
<td>Least Concern</td>
<td>Stable</td>
</tr>
<tr>
<td><em>Polypedates colletti</em></td>
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<td>Least Concern</td>
<td>Decreasing</td>
</tr>
<tr>
<td><em>Polypedates leucomystax</em></td>
<td>RHACOPHORIDAE</td>
<td>Least Concern</td>
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<td>Least Concern</td>
<td>Unknown</td>
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<tr>
<td><em>Rhacophorus appendiculatus</em></td>
<td>RHACOPHORIDAE</td>
<td>Least Concern</td>
<td>Decreasing</td>
</tr>
</tbody>
</table>

**Figure 4.1:** The complete list of species and individual found during the surveying period, including family, IUCN status and population trend (www.iucnredlist.org, 2010).

Both Indonesia and Borneo are species rich for herpetology and the potential for discovering new species is high. However the deforestation rates in this area are the...
highest in the world (Rowley et al., 2009) with the palm oil industry, mining industry and illegal logging the biggest threat to forests in Kalimantan. The results of this study show that the species richness is lower in the extremely disturbed forest (burnt new) which suggests that deforestation may have an impact on amphibian diversity. As seen in kerangas with stream habitat, small levels of disturbance are unlikely to affect species richness (Lempkert, 1999) therefore sustainable logging could occur in the area with little damage to populations. Bawan forest is currently not protected and its main threats are from the coal mining industry, illegal logging and fires. During the surveying period chainsaws, trucks and bikes were heard during the day and night, most likely by illegal loggers. If the logging continues and coal miners gain access populations may decrease and as several species are already listed as decreasing further falls may wipe out the populations in this area. During an orang-utan nest survey loggers were met on one of the transects. Old felled trees which hadn’t been removed were also frequently seen, especially in kerangas with stream. During the study scientists from a Japanese university came to assess the forest to see whether it would be suitable to be included in the REDD (Reducing Emissions from Deforestation and Forest Degradation) programme due to the high levels of carbon stored in the peat. This could be a potential way in which Bawan forest is protected. The high gibbon densities and orang-utan populations mean that the forest will be of great conservation interest to many. These animals can be flagship species and although the area may primarily be protected for these species in saving the forest the amphibian populations will be protected. This may be the only viable way as these amphibians will not be protected on their own as no species are in threatened IUCN categories.
Conclusions

The habitats of Bawan forest were different from one another in terms of species diversity, with some habitats showing statistically significant differences. Despite this each habitat had either different species unique to that habitat, unexplained large numbers of one particular species or evidence of breeding. These are all very interesting features which highlights the need for more amphibian studies in un-surveyed areas such as Bawan forest. The particular areas of interest at this site are the behaviour of breeding tree frogs, populations in the disturbed, burnt habitats, the potential for new species, especially in the mixed-peat swamp forest and sampling of the canopy to discover what species are there and to record their calls for future use. Species richness estimators concluded that at least a few more species inhabit Bawan forest and only follow up studies will determine which species these are.

MacKinnon’s List method as a technique to survey tropical amphibians is advantageous as it uses the time in the field more efficiently, opportunistic data can be included so it does not waste any data and field workers can be inexperienced as it observational work with little equipment. However this technique is biased against groups of individuals and it cannot produce viable relative abundance calculations or species diversity indices. As concluded by Muir (in press), MacKinnon’s List is a better method than visual encounter survey for a rapid assessment of an area. There is a growing need for baseline amphibian surveys in unstudied areas, especially in the tropics where many new species may exist; MacKinnon’s List method is a simple way of doing this.
The results of this study will hopefully highlight the amphibian diversity of Bawan forest showing that it is rich in species other than apes. These data along with the results of butterfly and moths surveys, orang-utan nest surveys and gibbon density surveys will hopefully gain this biodiverse area some protection from the illegal loggers and the encroaching coal miners.

Figure 5.1: *Kalophrynus* spp. found in mixed-peat swamp forest, only one individual encountered which could not be identified to species level (Picture by Iván Mohedano)
Acknowledgements

Firstly I would like to thank my supervisor Professor Roger Downie for helping me plan my project and for his invaluable input during my write-up. Thanks to Anna Muir who helped me plan my project and with my analysis, and to Joanne Smith who gave me advice on my methods. Thank you to Ross McLeod who showed me how to use EstimateS and to Stewart White who gave me access to Diversity Package and showed me how to use it. Thank you to the various people who helped with the species identification Alexander Haas, John Murphy, Robert Inger, Joanna Smith, Stefan T. Hertwig and Andre Jankowski.

Massive thanks to the Orangutan Tropical Peatland Project for letting me carry out my data collection as part of their long term project. Thanks to the project organisers Susan, Simon, Helen, Mark, Jess and Karen for giving me the opportunity and for all their support and advice in the field. My biggest thanks without a doubt is to Ivan who helped me beyond his call of duty and without his support and knowledge in the field my project would have fallen apart. A special thanks to Joanna for all your help with my data collection and for being a good student. Thank you to all the Indonesian field guides, without them I wouldn’t have been able to carry out my fieldwork. Special thanks to Ari,, Yanto, Hendri, Hairudin and Hanafi. A massive thanks to Lis and the Ibus for making all our amazing food. A huge thanks to all the other volunteers – Lilia, Sarah, Klara, Ellie, Kirsty, Charlotte, Charlie, Marie, Laura and Kath – who all help me so much with my data collection, and gave me so much support.
A big thank you to everyone who proof read my project, John Murphy, my dad, Iain, Marie, Lydia and Andy. Thanks to my parents, sister and gran for helping me fund my trip.

Figure 6.1: Author in the field with Polypedates colletti (Picture owned by Emily Waddell)


**Bibliography**


Appendix

All raw data is included in the enclosed CD.