Impacts of tropical rainforest disturbance on mammalian parasitism

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Project Declaration

The final design for this project was put together by Dr. Rob Ewers, Dr. Sarah Knowles, Phil Chapman MSc and myself. I collected all of the faecal samples from grids in the SAFE project, and Jessica Haysom MSc and Philip Chapman MSc collected the samples from the Maliau Basin Conservation Area. The other mammal data included in this analysis was collected by Jessica Haysom MSc and Philip Chapman MSc. The mammal trapping grids used were set up by Dr. Oliver Wearn. I processed all of the samples collected, and identified the parasite eggs, with the help of Dr. Sarah Knowles. I carried out all of the statistical analyses, with guidance from Dr. Sarah Knowles.
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ABSTRACT
Rainforest habitats in northern Borneo are becoming more and more fragmented due to the conversion of this land into oil palm plantation. This affects all of the ecological processes and species in these ecosystems, but the extent to which this is damaging is still being studied. Small mammals are abundant in the rainforests of Borneo and it is important to monitor their responses to habitat degradation due to their capacity to transmit diseases to humans. This study investigated the parasite burden of small mammals from different habitats within the SAFE project landscape. It was found that mammal communities are significantly different between different habitats, including, primary forest, logged forest and oil palm plantation. It was also found that the parasite burden of small mammals had a significant relationship with the trapping grid they were found in, with logged forest grids positively affecting the parasite burden of the mammal hosts found within them. These findings provide us with important baseline information on the parasites found in this area, as well identifying many areas for possible future research.

INTRODUCTION
Across much of Southeast Asia, areas of rainforest are being converted into agricultural land, dominated by oil palm (Elaeis guineensis) plantations. In the Sabah region of Malaysian Borneo, much of the original rainforest has been lost and converted into oil palm plantation. The Stability of Altered Forest Ecosystems (SAFE) project, a large-scale experiment that takes advantage of planned oil palm conversion, has been established to investigate the ecological impacts of modifying this tropical rainforest landscape (Ewers et al., 2011). The SAFE project landscape encompasses habitats from the full gradient of forest modification, from primary forest, to logged forest, to fragmented forest and finally oil palm plantation; allowing the monitoring of the effects of these changes on whole landscapes down to individual species (SAFE Project, 2010a). Now that the rainforests of Borneo provide a much more fragmented habitat, it is important to see the effects
of this on the species and ecological processes that occur there. A wide range of small mammal species are known to be present across this area. As much of the landscape has been disturbed due to the processes involved in logging and converting what was once primary forest to oil palm plantation, the distribution of small mammals is likely to have been dramatically altered. Theories suggest that small mammal abundance can increase in disturbed forest due to the increase in ground cover and understory vegetation (Lambert and Adler, 2000). So far in this area, studies have revealed that small mammal species richness and diversity may increase on a local scale, due to the increase in habitat heterogeneity after logging (Bernard et al., 2009). However, the conversion to oil palm plantation leaves the mammal community quite species poor, and can inhibit the dispersal of mammals. More recently, Cusack et al. (2014) found that native murid rodents are associated with less disturbed habitats, and more degraded habitats encourage invasive rodents, such as the black rat (Rattus rattus). The first part of this investigation will aim to explore the differences in the small mammal communities across the SAFE project landscape gradient, and it will be interesting to see if the same patterns emerge as in the other studies of small mammals in this area. Small mammals are known to be infected with a range of ecto- and endoparasites, some of which can pose a danger to humans, including Capillaria hepatica, Syphacia muris and Hymenolepis nana (Raj et al., 2009). It is important to build an understanding of the host-parasite interactions for this diverse group. This is due to the risk to humans from the parasites small mammals are known to harbour, and it is unknown at this point what the implications of deforestation are for small mammal’s parasite burden. It is thought that the stress placed on these mammals during the process of converting primary rainforest to oil palm could have great effects on their parasite burden. One of the current hypotheses, known as “the dilution effect”, suggests that more diverse ecological systems inhibit the spread of diseases. Conversely, it has also been hypothesized that high biodiversity indicates high numbers of potential hosts, “the amplification effect”, so there is more opportunity for diseases to spread (Randolph and Dobson, 2012). This is especially a risk in parasite-host systems where the
parasites are not host-specific. Civitello et al. (2015) found evidence of the dilution effect when looking at parasite-host interactions, and found that more diverse host communities inhibit the spread of parasites by altering the accessibility to susceptible hosts, and interfering with the parasite’s transmission pathway. Research by Wells et al. (2007) on small mammals in Northern Borneo has shown that individuals found in logged forest habitats had a higher species richness of endoparasitic nematodes than the individuals same species found in unlogged forests. Bordes et al. (2015) found that habitat fragmentation may not affect parasite species richness. As our study system spans across a habitat gradient from homogeneous to highly biodiverse, it will be interesting to compare parasite burdens from both ends of this spectrum and see if we attain similar results.

This is the first study of its kind to investigate the endoparasite burdens of the full assemblage of small mammals found in the live-trapping grids in this area of Borneo. Therefore, we are interested in discovering the types of parasites that are present in this area in general, as well as how the parasite-host relationships may be changed by the processes involved in habitat modification.

**Aims and Hypotheses**

We aim to investigate the effects of habitat disturbance on the parasite burden of small mammals in the Sabah region of Malaysian Borneo. This will involve looking at the animals caught in the live traps across the existing sampling grids in the different habitat types at the SAFE project, and at the Maliau Basin Conservation Area. This study will first look at the effects of habitat disturbance on the small mammal communities, as their composition can greatly affect parasite presence and transmission. Our expectation is that logging will increase the density of small mammals in remnant habitats that are not destroyed, and that surviving individuals will be stressed, which will lead to increased parasite burden and transmission among individuals. We will then go on to look at the factors that can be predictors of parasite presence and the effects these are having across the habitat gradient.

**METHODS**

**Study site**

The study site is located in the Sabah region of Malaysian Borneo, shown in
Figure 1. Data was collected from eight different trapping grids, across four sampling sites. These include two grids within the old growth control site of primary forest at the Maliau Basin Conservation Area, shown as OG2 in Fig.1, these grids have never experienced logging. One other control grid within the oil-palm plantation was also sampled, shown as OP2 in Fig.1, which was at one stage completely cleared of forest. The experimental fragments shown in Fig.1 were also used, including two grids within the 100-hectare fragment in block D, and two grids within the 100-hectare fragment and one grid within the 10-hectare fragment in block E. These fragments have experienced two logging cycles and represent a disturbed forest habitat.

Sample collection

We used a live trapping method using wire mesh tomahawk-style traps. 96 of these traps were put out at a time, placed at 48 points in each grid in a 12x4 formation, with two traps (A and B) positioned on the ground at each point.
Each trap was modified with a plastic sheet inserted into the base to stop the faeces from falling through the bottom of the wire mesh. A single piece of oil palm fruit was hooked into the interior of each trap as bait. The traps were left out in each grid for a period of seven days. Each trap was checked every morning. If the trap had made a successful catch it was collected in and the mammals were processed to take biometric data, (including age, sex, body measurements and weight), a count and sample of their ectoparasites (including mites, ticks and fleas), a tissue sample and a faecal sample. The traps were then repositioned, the mammals released, and the traps re-baited and reset.

**Sample analysis**

Faecal samples were analysed using passive faecal flotation technique. Sheather’s sucrose solution was added to each sample and ground in a fecalyzer™ to break apart larger debris and allow eggs to become dislodged. Samples were then left for 10 minutes to allow eggs to float to the top of the miniscus, where they would stick to a microscope cover slip. Samples were then analysed using a Leitz Dialux 20 compound microscope. All eggs were photographed using Infinity Analyze”™ software (Lumenera Corporation, 2007), and measurements were taken to facilitate identification. Parasite eggs were identified to the most specific taxonomic units possible and counted to establish the number of parasite eggs present in each sample. The number of eggs per gram of faeces for each mammal caught was calculated to establish overall endoparasite burden.

**Data Analysis**

To analyse the differences between the mammal communities, a linear mixed effects model, using package lme4 (Bates et al., 2015), was used to test whether the numbers of animals caught in the traps were significantly different between the grids and between the fragments. Two models were compared to test whether both the grid location and fragment had a significant effect on the number of animals caught using an ANOVA. A permutational MANOVA was then used to test whether there was a significant difference in the composition of the mammal communities caught within each of the fragments, using the adonis() function from the Vegan R package (Oksanen et al., 2015). Graphics were built to display these results using ggplot2 (Wickham, 2009).
To analyse the parasite communities within the hosts, the numbers of eggs per gram of faeces found in each of the different host species was tested using a quasipoisson generalised linear model (GLM). To test whether there was a significant difference in the types of parasites found in hosts from different mammalian families, a permutational MANOVA was used, again using the adonis() function from the Vegan package. A set of binomial GLMs were then used to test whether there was a significant difference in the presence of nematodes, cestodes, and Eimeria in all hosts across all of the trapping grids. Each of these models was tested for significance using $\chi^2$ tests and assessed for suitability by using diagnostic plots of deviance residuals.

To investigate more thoroughly the variables involved in predicting parasite presence, data from the two most abundant species were tested as we had a higher volume of samples to give these results higher statistical power. A binomial GLM was fitted to the data for Whitehead’s rats, testing the presence of endoparasite eggs against the age, sex, weight, number of ectoparasites and trapping grid of each individual. Another binomial GLM was then used to test which factors predict the presence of only nematode eggs, including age, sex, weight, number of ectoparasites and trapping grid of each individual. Another model was then fitted to the data, including the same variables but instead testing against the presence of cestode eggs, and then an additional model to test against the presence of Eimeria. The same four binomial GLMs were then fitted to the Red spiny rat data. Each of these models was tested for significance using $\chi^2$ tests and assessed for suitability by using diagnostic plots of deviance residuals.

**RESULTS**

**General patterns of host diversity**

Over a total of 56 trapping nights, 418 mammals were caught across the 8 trapping grids. These comprised 15 different species, including nine species of rat, three species of treeshrew and three species of squirrel. The total and relative abundances of each grid are shown in Figure 2. As can be seen in the first barplot in Fig.2, the number of mammals caught in each grid is very variable, with trapping grid being a highly explanatory factor of this ($\chi^2(7)=558.6, \ p<0.0001$). A permutational MANOVA was used to test for significant differences in the
mammal communities between the sampling sites (Oil Palm, Primary Forest, Fragment block D and Fragment block E) and the mammal communities in each of these were found to be significantly different \((F(4)=7.49, p=0.002)\).

Figure 2: The total and relative abundances of each species found in each of the trapping grids. Species found were Brown spiny rat \((Maxomys rajar)\), Common treeshrew \((Tupaia glis)\), Dark-tailed tree rat \((Niviventer cremoriventer)\), Ear-spot squirrel \((Callosciurus adamsi)\), Four striped ground squirrel \((Lariscus hosei)\), Large treeshrew \((Tupaia tana)\), Long-tailed giant rat \((Leopoldamys sabanus)\), Low’s squirrel \((Sundasciurus lowi)\), Muller’s giant Sunda rat \((Sundamys muelleri)\), Polynesian rat \((Rattus exulans)\), Red spiny rat \((Maxomys surifer)\), Slender treeshrew \((Tupaia gracilis)\), Small spiny rat \((Maxomys baeodon)\), Whitehead’s rat \((Maxomys whiteheadi)\).
**Parasite diversity and prevalence**

We collected a total of 211 faecal samples across the eight trapping grids. We found a total of 25 endoparasite morphotypes, including 17 nematodes, five cestodes and two Eimeria. Endoparasite eggs were present in 47.4% of samples. The number of eggs per gram of faeces was found to be significantly different between the host species ($\chi^2(13,197)=16850, \ p=0.047$), but this was not a strong relationship, and none of the individual levels of host species differed significantly from the intercept. This model explains 27.9% of deviance in the data. The eggs per gram of faeces for each host species are shown in Figure 3.

![Figure 3: A boxplot to show the median and interquartile ranges of the eggs per gram of faeces found for all parasite morphotypes found in all different host species. This data has been log transformed to account for the over dispersion of the data.](image-url)

A phylogenetic tree showing the presence of each type of parasite within the different host species can be seen in Figure 4. A permutational MANOVA test was done to see if the parasite communities of each host differ significantly between the three families of host (Muridae, Sciuridae and Tupaiidae). This was not found to be significant ($F(2)=0.8641, \ p=0.602$).
The prevalence of the three main types of endoparasite eggs (nematodes, cestodes and Eimeria) across the different host species in each of the fragments is shown in Figure 5. The first model tested whether host species and trapping grid were predictors of nematode presence. The trapping grid that the samples were taken from was found to be highly significant in determining the presence of nematode eggs ($\chi^2 (7,190)=28.66$, $p<0.001$), with higher prevalence of nematodes in samples taken from grids E100-2 grid and E10-1 grid. However, host species alone was not found to have a significant effect on nematode prevalence ($\chi^2 (13,197)=14.63$, $p=0.332$). This model was found to explain 80.3% of the deviance in the data. The same model was then used to test instead for the presence of cestode eggs, but neither trapping grid nor host were found to be significant (Grid: $\chi^2 (7,190)=3.62$, $p=0.82$, Host: $\chi^2 (13,197)=13.59$, $p=0.40$). The same model was then used to test for the presence of Eimeria, but this test showed that Eimeria prevalence did not differ significantly across the host species or trapping grids (Grid: $\chi^2 (7,190)=10.02$, $p=0.19$, Host: $\chi^2 (13,197)=12.21$, $p=0.51$).

Figure 4: A phylogenetic tree showing the relatedness of the species of mammal caught and all of the parasites that were found to infect them. Adapted from information from Achmadi et al. (2013), Lecompte et al. (2008) and Macdonald (2009).
Factors influencing parasitic infections in abundant host species

We had the highest number of samples from Whitehead’s rat (*Maxomys whiteheadi*) (n=45) and red spiny rat (*Maxomys surifer*) (n=47), and therefore decided to investigate further the factors that can be predictors of endoparasite infections. We used a binomial GLM to test how the probability of infection with any endoparasite was predicted by age, sex, weight, number of ectoparasites and trapping grid. For Whitehead’s rat, none of these factors explained significant variation in endoparasite prevalence (Age: $\chi^2(2,38)=1.551$, $p=0.461$, Sex: $\chi^2(1,37)=1.077$, $p=0.299$, Weight: $\chi^2(1,36)=0.219$, $p=0.640$, Grid: $\chi^2(5,31)=8.296$, $p=0.141$, Ectoparasites: $\chi^2(1,30)=3.62$, $p=0.388$).

The same factors were tested against the presence of nematode eggs. The number of ectoparasites was found to be significant, and have a positive relationship with the presence of
nematode eggs ($\chi^2(1,30)=5.4993$, $p=0.019$), but no other factors were significant (Age: $\chi^2(2,38)=0.5115$, $p=0.774$, Sex: $\chi^2(1,37)=0.1595$, $p=0.689$, Weight: $\chi^2(1,36)=0.6996$, $p=0.403$, Grid: $\chi^2(5,31)=4.1001$, $p=0.535$). The presence of cestode eggs was then tested against the same factors. Again, none of these predictors were significant (Age: $\chi^2(2,38)=2.3478$, $p=0.309$, Sex: $\chi^2(1,37)=1.4258$, $p=0.2325$, Weight: $\chi^2(1,36)=0.000$, $p=0.996$, Grid: $\chi^2(5,31)=2.2129$, $p=0.818$, Ectoparasites: $\chi^2(1,30)=3.64$, $p=0.056$). The presence of Eimeria was then tested against the same factors, again, the number of ectoparasites was found to be significant ($\chi^2(1,30)=7.49$, $p=0.006$), but no other factors were significant (Age: $\chi^2(2,38)=5.621$, $p=0.06$, Sex: $\chi^2(1,37)=1.296$, $p=0.255$, Weight: $\chi^2(1,36)=0.066$, $p=0.797$, Grid: $\chi^2(5,31)=6.99$, $p=0.221$).

The same tests were then run for the red spiny rat data. When testing the factors affecting the presence of endoparasite eggs, none of the factors were found to be significant (Age: $\chi^2(3,35)=4.51$, $p=0.211$, Sex: $\chi^2(1,34)=0.0018$, $p=0.966$, Weight: $\chi^2(1,33)=2.435$, $p=0.119$, Grid: $\chi^2(4,29)=3.26$, $p=0.515$, Ectoparasites: $\chi^2(1,28)=1.64$, $p=0.20$).
DISCUSSION

General Patterns of Host Diversity
The significant differences found in the small mammal communities across trapping grids are to be expected to some extent, especially between the different sampling sites, as they represent different habitat types. A somewhat surprising finding is the absence of invasive species of rat, such as *Rattus rattus*, as previous studies have found invasive species in the forests of northern Borneo (Cusack *et al.*, 2014). It is also interesting to see that the number of animals caught in each trapping grid differs significantly, when trapping effort is the same throughout. This is a finding that we expected, because although the old growth primary forest grids have a higher general biodiversity, the populations of these mammals are much less dense, and therefore it is not surprising that fewer mammals would be caught in primary forest compared to logged forest despite the trapping grids being of the same size. It has been found that small mammals are more abundant in disturbed habitats due to the denser understory and ground vegetation, a strong predictor of small mammal presence due to the increased cover and suitable habitat it provides (Lambert and Adler, 2000). Small mammals may also be more abundant in disturbed habitat due to the absence of predators that would otherwise control their population. Terborgh *et al.* (2001) found that rodents were 35 times more prevalent in forest fragments free of predators, which could also explain why we have found more small mammals in the disturbed forest fragments, however we do not have the data on predator presence in these areas to be able to draw strong conclusions from this.

The small mammal community composition also provides us with very interesting information. One of the most surprising findings was that the grid from the oil palm plantation had a more varied species composition than one of the primary old growth forest grids, and the same number of species as the other primary old growth forest grid. This is not what would have been expected, as the oil palm plantation is such a homogeneous habitat. This is also quite different from the findings of Bernard *et al.* (2009), who found a much more varied small mammal species assemblage in primary forest compared to oil palm plantation. Their study was also conducted in the forests of the Sabah region of Malaysian
Borneo and they found many of the same species. This shows how variation in geographic location can cause great differences in species composition in the same region, and it is therefore important to consider this when setting aside areas for conservation.

Although we have only collected 8 weeks worth of data, these findings could begin to shed light on which small mammal species can persist in a disturbed habitat, and which species are most susceptible to the changes. As this is part of a long-term study, will be able to contribute to illuminating small mammal community responses to habitat degradation and fragmentation.

**Parasite diversity and Prevalence**

The number of small mammals infected with endoparasites (47.4%), this is lower than found in a similar study by Wells *et al.* (2007), who found that 95% of small mammals were infected with endoparasites. These data were collected in a different area of Malaysian Borneo, which could account for the difference, but it is interesting to see such a large difference in infection rate when many of the species’ studied were the same. In other areas of the world, infections of rodents vary quite greatly, a study in China found 29.6% of a rodent population was infected with parasites (Yen *et al.*, 1996), and another study in Thailand found 86.8% infection rate (Na-Mue *et al.*, 1997). Therefore, although our infection rate seems low in comparison to Wells *et al.* (2007), it does fall within a normal range.

We found a fairly weak relationship between host species and total number of eggs per gram of faeces, which could suggest that other factors have more of an effect on parasite infections. We also found no significant relationship between the within-host parasite community and the host family. This goes against a theory put forward by Freeland (1983) that suggests that phylogenetic differences between host species, and also phylogenetic differences in parasite species, are factors that allow similar species to coexist in the same environment without competition. These results could occur due to a high number of parasite types compared to the number of faecal samples, which may not have been enough to show clear patterns of host specificity. It could also be that because these species all coexist together in the same habitats, they may acquire the same parasites from their environment. This idea would suggest that we would see a relationship...
between the number of eggs per gram of faeces and the trapping grid in which the host was found. We found this to be true of nematode eggs, our most prevalent type of endoparasite egg, but when we tested cestode eggs and Eimeria our results were not significant. This could be because, overall, we collected many more samples that contained nematode eggs, as 18 out of the 25 parasite morphotypes that we found were nematodes, so sheer numbers of samples could be contributing to this. We also found a significant positive relationship between presence of nematode eggs and grids E100-2 and E10-1, indicating that although E100-1 and E100-2 are next to each other, there are variations in parasite burden even in populations of mammals that are in very close proximity to one another.

The fact that these two grids had the highest host parasite burden neither support or contradict the theories of the dilution effect and the amplification effect. These two grids each contained 10 host species, which is the middle of our range of host species per grid (4-14). This shows that we found no evidence for the dilution effect, which would show that grids with higher species richness have a negative relationship with parasite burden, or the amplification effect, which would show that grids with higher species richness have a positive relationship with parasite burden.

**Factors influencing parasitic infections**

In order to look at the factors that contribute to endoparasite burden, we concentrated on abundant species for which we had good parasite information, Whitehead’s rat and the red spiny rat. Our results from modelling their endoparasite presence data show that prevalence of nematodes, cestodes and Eimeria did not vary significantly across trapping grids, according to host age, sex, weight or the number of ectoparasites. When testing the red spiny rat data alone, host age, sex, weight and ectoparasite number did not have a significant relationship with the presence of any type of endoparasite. The number of ectoparasites present per individual was the only factor that was significant and had a positive relationship for predicting nematode and Eimeria infection in Whitehead’s rat. This is similar to findings from Wells *et al.* (2007), who found no relationship between host age, sex and weight and the number of nematode species present in a host. It could be that
certain host characteristics do not determine parasite infection, and instead the rate of parasite infection is influenced by the ease of transmission between hosts, as found by Bordes et al. (2015). However, a study by Schalk and Forbes (1997) found that there were male biases in parasitism rates, they thought this was due to the effect that female hormones have on the immune response, so certain host attributes can affect parasite infections. Our finding that the number of ectoparasites had a significant relationship with endoparasite presence could indicate that endoparasites may be more common in individuals who have been immuno-compromised by the presence of ectoparasites. Or it could be possible that ectoparasites transfer endoparasites into their host species. Unfortunately this relationship between ecto- and endoparasites has not been well studied, but could be worth considering for future research.

Limitations and ideas for future research
This investigation did experience some methodological caveats. The floatation solution can cause problems with faecal egg count. Endoparasite eggs could have been missed from samples due to not being able to float to the top of the solution. Storage of samples in a tropical climate was also difficult and may have affected the quality of the samples due to difficulty with refrigeration. As there was a large variety of species found and also a great diversity in parasite eggs, having large enough samples sizes to incorporate into statistical analysis was difficult. Ideally, we would have analysed the differences in the parasite burden of all mammals found within the different fragments, but due to low numbers of each type of parasite found, and no mammal species being found consistently in all of the sample sites, there were too many variables to perform a reliable analysis on this. We were also unable to analyse how parasite burden changes along a habitat degradation gradient, due to low numbers of samples from both the primary forest control and the oil palm control.

Due to the time constraints of this fieldwork, it was difficult to obtain as many samples as we hoped. Therefore, ideas for future work would include sampling more trapping grids, and resampling the same grids repeatedly over several years to be able to establish how parasite burden is changing over time as the landscape
becomes increasingly degraded. The methods for collection of faecal samples could also be improved to ensure faeces do not fall out of the traps during transit. As mentioned earlier, studying the affect that ectoparasites have on the immune system of small mammals could also shed interesting light on how this might affect the likelihood of endoparasite infection. It could also be interesting to look at blood parasites in the future as they are also known to carry zoonotic diseases; sample collection for this could be combined with the tissue samples currently being collected. It could also be interesting to look into methods for sampling small mammals living higher off the ground in different levels of the canopy, to see if they are affected by habitat modification in the same way.

Conclusions
Overall, this investigation has provided us with some baseline information on the types of parasites found in the small mammals in the rainforests of the Sabah region of Borneo. We have seen that many parasites are present in this area, and host sharing is very common among these parasites. Although we cannot draw strong conclusions from our data due to small sample sizes, this study has shown that there are differences in the mammal communities and host-parasite relationships between the different sample sites, which may be a results of habitat fragmentation and degradation, and can open up the possibilities for future research to see the effects this could have on the wider ecosystem.

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