A survey of endoparasites in endangered Bornean elephants

Elephas maximus borneensis in continuous and fragmented habitat

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Abstract

Habitat loss and fragmentation are understood to be primary causes of biodiversity loss. There is also increasing recognition of disease as a threat to biological diversity. However, few studies examine interactions between the threats of habitat loss and disease or the links between ecosystem and animal health. Indeed even basic baseline data on pathogens and parasites is lacking in most if not all wild animals.

This study investigates links between ecosystem and animal health by examining endoparasite infection in wild Bornean elephants *Elephas maximus borneensis* in fragmented versus continuous habitat in Sabah, Malaysia. Over one hundred faecal samples were collected from elephants in the Lower Kinabatangan Wildlife Sanctuary comprising of small patches of highly fragmented forest and Tabin Wildlife Reserve, the largest continuous reserve in Sabah.

Endoparasites were found to be ubiquitous in endangered Bornean elephants. Using a special modification of the McMaster method, endoparasites classed as trematodes, cestodes and nematodes were identified. There were significant differences in parasite prevalence, load and diversity between fragmented and continuous habitat. Variations could be attributed to links between ecosystem and animal health and the effects of habitat loss and fragmentation on disease dynamics. Most notably, the prevalence and load of strongyle nematodes was significantly higher in fragmented compared to continuous habitat suggesting that habitat fragmentation is associated with increased incidence of disease spread by faecal-oral transmission.

This study provides not only the first catalogue of endoparasites in wild Bornean elephants but also highlights links between ecosystem and animal health. Findings add to a growing body of evidence which indicates that habitat loss and fragmentation influences wildlife disease dynamics. In light of this interaction, this study provides the basis to develop novel tools to monitor and manage wildlife and landscapes threatened by anthropogenic habitat loss and fragmentation.

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Acronyms and definitions

DGFC  Danau Girang Field Centre
epg  Eggs per gram of faeces
KWS  Kinabatangan Wildlife Sanctuary
rpm  Revolutions per minute
spg  Specific gravity
SWD  Sabah Wildlife Department
TWR  Tabin Wildlife Reserve

Endoparasites  Internal parasites

Habitat  The transformation of an expanse of habitat into a number of smaller patches isolated from one another by a landscape matrix unlike the original habitat

Habitat loss  The complete elimination of a localised or regional ecosystem resulting in total loss of biological function

Helminths  Parasitic worms

Prevalence  The number of positive cases present at a certain time as a percentage of the total number sampled

Parasite load  A numerical count of parasite eggs expressed as eggs per gram. Also known as faecal egg count, parasite infection intensity or egg density

Parasite diversity  A numerical count of the number of different parasite types observed
1. Introduction

1.1 Problem statement

Global biodiversity is under threat. Current extinction rates are up to a thousand times that of historical background rates (Millennium Ecosystem Assessment, 2005) in what has been dubbed the sixth global mass extinction (Larsen, 2004). Habitat loss, associated with accelerated human population growth and resource use, is perhaps the most significant driver of diminishing biodiversity (McCallum, 2002).

Habitat loss and fragmentation have a severe impact on wildlife population viability and the persistence of endangered species (Acevedo-Whitehouse and Duffus, 2009). A prime example is the endangered Bornean elephant *Elephas maximus borneensis*.

Habitat loss and fragmentation are the principal threats to the survival of Bornean elephant in Sabah, Malaysia. Habitat conversion in Sabah reached a peak in the 1980s and 1990s and by the year 2000 over a million hectares of elephant habitat, representing 14% of the state’s entire land area, was converted for agriculture mainly oil palm (Sabah Wildlife Department, 2012). It is estimated that by the year 2022, 98% of primary forest in Borneo will have been cleared (UNEP, 2007).

Elephants are particularly vulnerable to habitat loss and fragmentation. As large bodied mammals with high resource and space requirements, they belong to a high risk group for anthropogenic extirpation. Reductions in species ranges have been cited as major contributing factors to the decline of large mammals globally (Barnosky *et al*., 2011). The Indomalaya area in particular, only holds 1% of the large mammals it historically held and has been identified as an urgent conservation priority (Morrison *et al*., 2007).

Bornean elephants fulfil further criteria for high extinction risk: a small population with a restricted range and patchy distribution (Smith *et al*., 2009). In order to mitigate this risk and conserve Bornean elephants *in situ*, it is essential that interacting threats to species survival are understood in context, not just in isolation.
A conservation medicine or One Health approach which investigates links between ecosystem and animal health provides the basis for a holistic approach to better understand, assess, monitor and improve the status of individuals, populations and habitats at risk (Aguirre, 2002).

Ecosystem and animal health are inextricably linked. Anthropogenic habitat loss and fragmentation can influence wildlife disease dynamics via shifts in wildlife populations and changes in disease ecology. These shifts can be mediated by a myriad of mechanisms including hindering animal movement, impeding gene flow (Coulon et al, 2004), promoting edge effects (Chapman et al, 2006b), introducing environmental contamination (Deem et al., 2001), altering the ecology of intermediate hosts (Page et al., 2001), changing host population size and density (Mbora and McPeek, 2009), limiting nutrition (Chapman et al, 2006a), facilitating contact and conflict with people (Nelson et al., 2003) and subjecting animals to psychological and physiological stress and affecting immunocompetence (Schwitzer et al, 2010). Yet there is insufficient attention given to these links in wildlife conservation (Thompson et al., 2010).

Even the most basic baseline data on pathogens and parasites is lacking in most, if not all, wild animals (Matthews, 2009) and the nature of interactions between disease and habitat disturbance remains poorly defined (Gillespie and Chapman, 2008; McCallum and Dobson, 1995). These knowledge gaps demand urgent attention to characterise the effects of habitat loss and fragmentation and enable an effective holistic One Health approach to conservation.

This study addresses a significant knowledge gap in conservation science by investigating potential interactions between two key threatening processes, habitat loss and disease. This is a step towards a One Health approach, bridging ecosystem and animal health in the face of anthropogenic habitat loss and fragmentation.
1.2 Aims and objectives

This project aims to evaluate links between habitat loss, habitat fragmentation and animal health by comparing the prevalence, load and diversity of endoparasites in endangered Bornean elephants in highly fragmented versus continuous forest habitat in Sabah, Malaysia.

Objectives

- To develop and trial a practical protocol to screen endoparasites in free ranging wild Bornean elephants
- To profile the endoparasites of Bornean elephants
- To assess patterns of endoparasite infection in Bornean elephants in fragmented and continuous habitat

The hypotheses to be tested:

- The prevalence, load and diversity of endoparasites in Bornean elephants vary with the degree of habitat fragmentation
- Endoparasites are more prevalent, present in greater numbers and are more diverse in fragmented compared to continuous habitat
2. Background

2.1 Bornean elephants

The Bornean elephant *Elephas maximus borneensis* is the most recently classified Asian elephant subspecies and is listed by the IUCN as endangered (A2c) (Choudhury *et al.* for the IUCN, 2008). The main threats to Bornean elephants are habitat loss and fragmentation. Human elephant conflict is also a growing concern as elephants are forced to eke out an existence in remaining habitat surrounded by palm oil plantations and human settlements (Sabah Wildlife Department, 2012).

*E. maximus borneensis* are morphometrically distinct from their relatives on the mainland having larger ears, longer tails, straighter tusks and a more rounded body shape (Othman *et al.*, 2008). Arguably, they are also genetically distinct having evolved in isolation in Sabah and as such constitute an evolutionary significant conservation unit (Fernando *et al.*, 2003). Regardless of its origins, the Bornean elephant is a conservation priority as an iconic flagship and umbrella species carrying out vital ecosystem services (Campos-Arceiz and Corbett, 2011).

The most recent survey estimates by dung count that there are approximately 2040 elephants (95% CI: 1184-3652) confined to significantly degraded and disconnected habitat in the northeast of Sabah, Malaysia (Alfred *et al.*, 2010).

2.2 Elephant endoparasites

This study focuses on endoparasites, specifically extracellular helminth macroparasites including trematodes, cestodes and nematodes in contrast to ectoparasites, which reside on external surfaces of the host or intracellular microparasites which live inside host cells.

Research on endoparasites of Asian elephant is limited to only a handful of published studies, the majority of which focus on captive populations (McLean *et al.*, 2012). No
previous studies unequivocally include endoparasites of Bornean elephants, captive or wild.

Drawing mainly on data from studies on captive *E. maximus*, the most comprehensive catalogue of elephant endoparasites currently available is compiled in Fowler and Mikota’s *Biology, Medicine and Surgery of Elephants*, the definitive text on Asian elephant veterinary medicine. The catalogue includes species of strongyles (helminth nematodes) of the genus: *Murshidia*, *Quilonia*, *Amira*, *Decrusia*, *Equinurbia*, *Choniangium*, *Bathmostomum*, *Grammocephalus* and *Parabronema*. Digenean trematode flukes are represented by species of *Fasciola* and *Pseudodiscus* and cestode tapeworms by *Anoplocephala* (Fowler and Mikota, 2006).

Surveys of endoparasites in currently free ranging wild Asian elephants are uncommon in the literature. Perhaps the most detailed and long term study is by Chandrasekharan et al. (2009). Over a thirty year period, Chandrasekharan and his colleagues at the Kerala Agricultural University Elephant Research Centre surveyed captive and wild elephants. Using unspecified parasitological methods, they found 21 different species of helminths including those listed in the definitive catalogue. The prevalence of helminths was reported to be high; all captive elephants and 38% of wild elephants harboured strongyles (Chandrasekharan et al., 2009). Using a sedimentation-flotation method, a similar inventory of endoparasites was reported by Vidya and Sukumar (2002) in their study on free ranging wild elephants in southern India.

Closer to the current study sites, surveys of Asian elephant endoparasites have been conducted but none unequivocally include wild Bornean elephants. Fernando and Fernando (1961) report an inventory of Asian elephant helminths from the collection of the Parasitology Department at the University of Malaya, Singapore. It is unclear whether these specimens originated from captive or wild *E. maximus* from Peninsula Malaysia or *E. maximus borneensis* from Sabah as samples were labeled only as ‘elephant in Malaya’. They report five species of nematodes: *Murshidia murshida*, *M. falcifera*, *Quilonia renniei*, *Equinurbia sipunculiformis* and *Choniangium epistomum* and one species of trematode *Pjenderius papillatus*. 
As part of their Elephant Healthcare Program, The Veterinary Society for Sumatran Wildlife Conservation (VESSWIC) undertake regular screening, of captive Sumatran elephants *E.maximus sumatrensis* using unspecified flotation and sedimentation methods. The most frequent endoparasites reported are strongyles and *Fasciola* and *Paramphistome* trematodes (Stremme *et al.*, 2007). A recent survey by Muriyani *et al.* (2008), using the McMaster method, similarly found a high prevalence of trematodes (64.86%) in 37 captive Sumatran elephants at the Sumatran Elephant Training Centre. A low prevalence of ascarid roundworms (2.7%) was also reported. Similar parasite genera were found by Matsuo and Suprahman on *post mortem* examination of three deceased Sumatran elephants (Matsuo & Suprahman, 1997).

In summary, though the literature is scant, there appears to be a general consensus on the profile of Asian elephant endoparasites. Depending on the method used, a parasite survey of Asian elephants might expect to find the majority of eggs are strongyles, mainly cyathostomids including *Murshidia* and *Quilonia* as well as ancylostomid hookworms such as *Bunostomum* species. *Fasciola* trematodes may be prevalent. Cestodes may be seen but it is frequently not possible to identify tapeworm eggs in faecal flotation. Ascarid roundworms such as *Toxocara* species may be encountered but they are considered rare (Fowler and Mikota, 2006).

### 2.3 Endoparasites and elephant health

Endoparasites are generally not considered life threatening in healthy adult animals. However, changes in disease dynamics due to altered host, environment or pathogen factors can result in clinically significant endoparasite infection. Even normally innocuous parasites may diminish individual and population health, reproductive success and fitness when combined with other threats including concurrent disease, malnutrition, significant stressors, debilitated immunocompetence and decreased genetic variability (Lloyd, 1995). Decreased genetic variability reduces a host population’s ability to cope with infectious disease and parasitism by decreasing average resistance and or reducing variations in adaptive traits (Scott, 1988; Luquet *et al.*, 2011).
Clinical endoparasite infections have been reported in Asian elephant. Chandrasekharan et al. (2009) and Saseendran et al. (2004) report that gastrointestinal nematode infection has been associated with frequent clinical illness including colic, diarrhoea and dependent oedema in elephants managed in captivity in Kerala, India. In the extreme, case reports exist of elephant fatalities associated with endoparasites. A 14 year old Indian elephant imported from Singapore to Australia was reported to be anaemic, emaciated and exhibiting “profuse diarrhoea” and passing “buckets of worms” just days before his death. On post-mortem examination, the cause of death was cited as gastric abscession by the stomach worm Parabronema indicum concomitant with severe hookworm Bathmostomum sangrei (Sutherland et al., 1950). Similarly, a one year old elephant died after being shipped from Thailand to the USA and at necropsy was found to have a significant burden of B. sangrei, a variety of other nematodes and trematodes (Mikota et al., 1994). Clinical disease in Asian elephants associated with Fasciola has also been described in some detail in the literature with clinical signs including severe submandibular and ventral oedema (Mikota et al., 1994).

2.4 Endoparasites and conservation

Endoparasites are significant to conservation in terms of biodiversity, ecology and evolution. Biodiversity conservation is focused on sustainably managing the variety of life on Earth and parasite species constitute the majority of biodiversity with more species employing parasitism than any other life strategy (Lafferty et al., 2006; Nichols and Gomez, 2011). Conservation is concerned with maintaining ecosystem structure and function and parasites are integral to this, with a greater number of trophic connections between parasites and hosts than between predators and prey (Marcogliese, 2004; Lafferty, 2008). In terms of conserving significant evolutionary links, there are perhaps no more important life forms than parasites (Ridley, 1994).

Parasites can shape presence absence, distribution, population size and viability by affecting host survival, morbidity and fecundity directly by causing pathology or indirectly for example via host debility (Gulland, 1995; Hechinger and Lafferty, 2005; Nichols and Gomez, 2011). In this way, parasites have the potential to affect populations, especially
small populations of endangered species (Gregory and Hudson, 2000). A stark illustration of this is the giant panda, the global pin up for endangered species conservation. Mortality data analysis has revealed that the current most significant threat to wild panda is the result of interaction between habitat loss and parasitism. In the period 2001 to 2005, visceral larval migrans of the ascarid nematode *Baylisascaria schroederi* is said to have been responsible for $50 \pm 10.2\%$ (12/24) of reported panda deaths and it is hypothesised that this was related to augmented faecal oral transmission with increased population density as panda have been forced to live in closer proximity in fragmented patches of remaining bamboo forest (Zhang et al., 2008).

Despite their relevance to biodiversity, ecology and evolution, parasites have been neglected in conservation science. A recent analysis of English language conservation textbooks published between 1970 and 2009 revealed that 72% made no mention of parasites or portrayed them only in one dimension as a threat to conservation efforts (Nichols and Gomez, 2011). It has been said that “most conservation biologists throughout the world today are probably oblivious to the relevance of parasites” (Gomez, 2012) and there are growing calls for research to improve our understanding of parasites in conservation (Lafferty, 1997; Gregory and Hudson, 2000; Marcogliese, 2004; Whiteman and Parker, 2005; Gillespie and Chapman, 2006; Gomez et al., 2012).

In mid-2012, for the very first time, a chapter in a conservation text was dedicated to links between habitat loss, habitat fragmentation and infectious disease ecology. Suzan et al. (2012) include a synthesis of papers to January 2011 which examine interactions between habitat loss, habitat fragmentation and the infection dynamics of ectoparasites, endoparasites, bacteria, viruses and protozoa. Using the Science Citation Index, which covers over 3700 of the world's leading scientific and technical journals, only 33 papers fit the search criteria confirming a significant knowledge gap.

This study seeks to address a neglected area of conservation science from the perspective that ecosystem and animal health are linked and monitoring parasites can be an indicator of both (Lafferty, 1997; Marcogliese, 2005; Howells et al., 2011). Parasites can serve as an effective, practical, non-invasive, early warning system for the health of individual
hosts, host populations and the ecosystems of which they are a part because environmental changes impact upon both hosts and their parasites (Lafferty, 1997; Marcogliese, 2005). Endoparasites make good candidates for a study on the interactions between habitat fragmentation and disease dynamics in free ranging wildlife. Not only are they a practical conservation tool as they can be diagnosed using logistically feasible methods, they are also an effective indicator reflecting individual and population fitness as well as biotic and abiotic environmental changes such as those associated with anthropogenic habitat loss and fragmentation (Marcogliese, 2004).

The interaction between anthropogenic habitat disturbance and parasitism is likely to be complex. To date, evidence from wild populations of various taxa indicates that habitat loss and fragmentation have the potential to affect wildlife health in a variety of seemingly contradictory ways. For example, fragmentation may decrease parasite prevalence by isolating individual hosts thus decreasing density to a level below that required for transmission. Conversely, transmission rate may be increased in fragmented habitat as individuals are forced to live in smaller patches in closer proximity (Altizer et al., 2007).

Endoparasites can differ significantly between populations in fragmented versus continuous habitat (Gillespie and Chapman, 2008; Schwitzer et al, 2010). For example, the prevalence and load of the roundworm nematode Baylisascaris procyonis was significantly higher in racoon in fragmented versus continuous habitat in Indiana USA due to altered ecology of white footed mice, the intermediate host (Page et al, 2001). Endoparasite prevalence in howler monkeys in Mexico has been found to be almost twice as high in fragmented compared to continuous habitat (Trejo-Macias et al., 2007) and in several studies on colobus monkeys in Uganda, Gillespie and Chapman have showed that patterns of habitat disturbance including selective logging and forest fragmentation have a significant impact on host parasite relationships (Gillespie et al., 2005; Gillespie and Chapman, 2006; Gillespie and Chapman, 2008). Schwitzer et al. (2010) established that high parasite prevalence is associated with a high degree of habitat fragmentation in blue-eyed black lemurs in Madagascar and concluded that endoparasites have the potential to be used as indicators of stress in wildlife threatened by habitat fragmentation.
2.4 The study sites

This study was carried out in the Malaysian state of Sabah. The climate is equatorial with average annual precipitation of 2000 to 4000mm and a typical daily temperature of 30°C throughout the year (Payne and Francis, 2005). These conditions may be seen as ideal for endoparasites as they promote hatching rate and larval development (Roberts et al., 2009). Indeed, conditions in Sabah are ideal for a great diversity of life. It is home to some of the richest tropical moist forest on Earth (Olson and Dinerstein, 2002) and one of the last strongholds of Bornean elephant (Sabah Wildlife Department, 2012). Despite ongoing threats including habitat loss and human wildlife conflict, an extraordinary wealth of species has been identified here with an average of three new species classified every month between 1994 and 2004 (Rautner et al., 2005). The degree of endemism is also extremely high with over 45% of mammal species and 60% of plants not found anywhere else on Earth (Conservation International, 2007). The wider Southeast Asian region sees the overlap of four global biodiversity hotspots and in addition to elephants, remaining endangered large mammal species include Bornean orangutan Pongo pygmaeus, Sumatran rhinoceros Dicerorhinus sumatrensis Malayan sun bear Helarctos malayanus and Sunda clouded leopard Neofelis diardi (Payne and Francis, 2005).

Threats to Sabah’s biodiversity have been mounting since logging and land conversion began in the 1950s. Habitat loss accelerated to unparalleled rates during the 1980s and 1990s to feed insatiable global demand for tropical timber and palm oil. This trend continued, with time series analysis revealing that between 1991 and 2008, an astounding 31% of total forest cover was cleared (Johansen and Johansen, 2011).

The distribution of Bornean elephant is limited to the remaining lowland forest in the northeast of Sabah in four managed elephant ranges: 1) Lower Kinabatangan Wildlife Sanctuary, 2) Tabin Wildlife Reserve, 3) Deramakot, Ulu Segama and Kalabakan and 4) Ulu Kalumpang/Tawau Hills (Sabah Wildlife Department, 2012). This study was carried out in Kinabatangan and Tabin.
The Lower Kinabatangan Wildlife Sanctuary is a mosaic of primary to secondary lowland dipterocarp forest in a matrix landscape with significant ongoing human impact including villages, small scale agriculture, oil palm plantations and a busy tourism industry. The total area of the sanctuary is 400 km² but this is comprised of highly fragmented patches which lack connectivity. Estimates of available elephant habitat within the Kinabatangan vary from 138.15 km² (Alfred et al., 2010) to 184.23 km² (Estes et al., in press) which equates to just 35 to 46% of the total area. Available habitat is further reduced during the wet season when flooding limits elephant habitat to just 61.75 km² (Estes et al., in press). Elephants must share these small patches of remaining habitat with a growing human presence. They are exposed to daily anthropogenic stressors including tourist vessels and constant human and vehicular traffic in and around the plantations. Incidents of human elephant conflict are increasingly common as elephants are forced to cross through plantations and settlements to reach viable habitat (Estes et al., in press). There is an estimated total population of 298 elephants (95% CI: 152-581) in Kinabatangan at a density of 2.15 elephants per km² (95% CI: 1.31-2.99), the highest density of elephants in Sabah (Alfred et al., 2010).

The second study site, the Tabin Wildlife Reserve has a much lower density of elephants at 0.6 individuals per km² (95% CI: 0.32-0.88) but a larger estimated total population of 342 elephants (95% CI: 152-774) (Alfred et al., 2010). Tabin, a former logging concession, was gazetted in 1984 and is now Malaysia’s largest nature reserve consisting of primarily early to mid-secondary growth dipterocarp forest with scattered pockets of remnant primary forest (Dawson, 1993). The 1200 km² reserve includes 569.1 km² (approximately 47%) of key habitat and here elephants potentially have a greater degree of choice to distance themselves from human activity compared to elephants in Kinabatangan. However, it must be noted that Tabin is completely surrounded by palm oil plantations including one of the largest plantations in South-east Asia, into which elephants occasionally cross because plantations now occupy the best part of preferred elephant habitat, lush lowlands with river access. In the months prior to sampling, it is assumed that the elephants in Tabin spent their time in the secondary and remnant primary forest within the reserve as they had not been sighted since August 2011. At the time of collection, elephants were present near base camp and some were crossing into plantations at night.
3. Materials and methods

3.1 Sample collection

Opportunistic sampling of elephant faeces was carried out in the Lower Kinabatangan Wildlife Sanctuary (5°18’N to 5°42’N, 117°54’E to 118°33’E) and Tabin Wildlife Reserve (5° 10’N to 5°15’N, 118°30’E to 118° 45’E) during expeditions from April to June, 2012. In the Kinabatangan, elephant faecal samples were collected while shadowing elephant researcher Ms Nurzhafarina Othman. Here, elephants were located with the aid of Ms Othman’s expertise as well as GPS as the Sabah Wildlife Department and Danau Girang Field Centre have fitted several individual wild elephants with GPS collars. In Tabin, there were no collared elephants. Here, the expertise of experienced wildlife rangers was harnessed to track elephants via reports from local people and indirect signs including footprints and evidence of feeding.

Elephants were tracked on foot. Once located, time was allowed to elapse until the elephants moved away to a safe distance. Freshly deposited faeces were identified by appearance including colour, consistency and observed insect activity. Boluses were then measured and fresh samples collected from boluses of different sizes where they were situated at a distance from one another. This was done to minimise duplication and increase the reliability of prevalence estimates.

Core samples from the centre of the bolus were collected to decrease the risk of contamination by soil nematodes (Mikota, personal communication). This does not introduce bias because distribution of eggs through a bolus has been shown to be homogenous (Vidya and Sukumar, 2002). Samples were collected in pre-prepared polyethylene specimen containers containing 95% ethanol (Modry, personal communication, Appendix A). Several boluses in the dung pile were sampled in this way to ensure the sample was representative.
3.2 Sample analysis

Methods for parasitological surveys are largely chosen based on the types of parasites one expects to find in the target species. As this was the first study on endoparasites in Bornean elephants, selecting a method was challenging. The McMaster method was selected for this study based on the small number of parasitological studies on other Asian elephant subspecies, accepted methodologies in veterinary parasitology and specific advice from experts (Gillespie and Chapman, 2006; Emery, personal communication; Gillespie, personal communication; Modry, personal communication; Fox, personal communication; Fox, 2012; Harris, personal communication; Spratt, personal communication).

The McMaster method is considered to be a practical field technique giving a reliable indication of parasite prevalence and load. Egg recovery from Asian elephant faeces is considered high using faecal flotation methods such as the McMaster method (Watve, 1993) and it has been used effectively in previous work on endoparasites in Sumatran elephants (Muryani et al, 2008).

Centrifugation was employed to optimise parasite detection (Blagburn and Butler, 2006; Appendix B) and a special modification was incorporated to increase the sensitivity of the McMaster method from 50epg to 10epg (MAFF, 1986; Appendix C).

Eggs were observed using an Omax digital binocular compound light microscope (Model MD827S30 series). Photographs of parasite eggs were captured and measurements taken using the microscopic imaging software ScopeImage 9.0 H3D.

Parasite load was recorded as eggs per gram of faeces (epg). Parasites were identified based on egg morphology and morphometry, using professional training, parasitology texts and expert advice (Fox, personal communication). As strongyle eggs all have similar morphology, egg morphometry is the most direct method to diagnose strongyle infections (Georgi and McCulloch, 1989). However, egg measurements of different strongyle species overlap. Hence, discriminant analysis of strongyle egg morphometry was undertaken to approximate strongyle species diversity (Fox, personal communication).
Samples were allocated a parasite diversity score (out of a total of 3) based on the number of different phyla of parasites observed with a maximum score of 3 indicating the presence of trematodes, cestodes and nematodes.

All data were collated in Microsoft Excel 2010 (Appendix D)

3.3 Spatial analysis

Using a handheld GPS (Garmin GPS MAP 60CSx), the latitude and longitude of locations where samples were collected were recorded. These locations were mapped using ArcMap10.

3.4 Statistical analysis

Statistical analyses were performed using Microsoft Excel 2010 and R 2.14.1 (Appendix E)

A chi-squared test was applied to compare parasite prevalence and diversity between fragmented and continuous habitat. This test was appropriate as samples could be allocated to one of two categories, positive or negative and came in the form of frequencies.

The Mann Whitney U test, also known as the Wilcoxon Mann-Whitney test, was employed to compare parasite load between sites and parasite load with immediate versus delayed fixation. This test was appropriate to compare two equal groups of independent data where normal distribution could not be assumed.

The Spearman rank correlation coefficient was used to investigate correlation between parasite load and bolus diameter and parasite load and weight of sediment. This rank correlation was appropriate as there were two variables for each sample, the variables were continuous and could be ranked in a meaningful order, the relationship between these variables was not necessarily linear and the data was not necessarily normally distributed (Dytham, 2003).
3.5 Other comparisons

In addition to comparisons between parasite prevalence, load and diversity between fragmented and continuous habitat, the following investigations were carried out as asides to examine confounders which may affect parasite load including bolus diameter as a proxy for age, sediment weight and timing of fixation. Intra-individual variation was also explored via the repeat sampling of a mature bull elephant.

**Bolus diameter and parasite load**

Age can influence patterns of parasite infection. Parasite load has been shown to vary with age, possibly associated with differences in innate immunity (de Coster et al., 2010). Considering samples could not be individually identified in this study, the most practical method to estimate the age of elephants sampled was bolus diameter. Diameter is preferred over circumference because it is simple and quick to perform in the field. This is especially important when time is of the essence while collecting samples when surrounded by wild elephants (Reilly, 2002). Studies on African savannah (Morrison et al., 2005) and Sumatran elephants (Reilly et al., 2002) have established that bolus diameter is a suitable measure for estimating age. Up to a point, mean bolus diameter increases with age (Morrison et al., 2005). It must be noted that age estimation by bolus diameter has yet to be formally validated in Bornean elephants.

In this study, covariation of parasite load and bolus size (as a rough proxy for age) was investigated using Spearman’s rank correlation following a similar approach to Watve who performed this comparison in his study of over 200 Indian elephants (Watve, 1993).

Bolus diameter was also compared between fragmented and continuous habitat using the Mann Whitney U test to assess potential uneven age distribution as a confounder.

**Sediment weight and parasite load**

Considering that a variable amount of sediment was gleaned from different samples and this may have affected egg counts, correlation between sediment weight and parasite load was investigated using the Spearman rank correlation.
Sediment weight was also compared between fragmented and continuous habitat using the Mann Whitney U test to assess potential uneven sediment weights as a confounder.

**Timing of fixation**
Delay between time of collection and return to the field laboratory usually precluded examination of unfixed samples. However, during the course of this study the occasion arose to collect seven fresh samples and return to the laboratory that same day. This provided the opportunity to conduct a trial experiment to assess the effect of timing of fixation on parasite load by comparing immediate versus delayed fixation. Using the Mann Whitney Wilcoxon test, the egg counts of samples fixed in ethanol immediately at the time of collection were compared to counts from the same samples fixed approximately twelve hours post-collection.

**Repeat sampling of a mature bull**
On two separate occasions, 24 April and 22 May, a mature bull identified as ‘Elvis’ was observed defaecating and samples collected shortly thereafter to investigate intra-individual variation in parasite load and diversity.
4. Results

A total of 104 faecal samples were collected from wild Bornean elephants in the Lower Kinabatangan Wildlife Sanctuary \((n=52)\) and Tabin Wildlife Sanctuary \((n=52)\) (figure 1).

![Map of sample collection locations](image)

**Figure 1. Map of sample collection locations** Lower Kinabatangan Wildlife Sanctuary (LKWS) and Tabin Wildlife Reserve (TWR) including layers created by Danica Starke and Luke Evans and georeferencing from an image provided by Penny Gardner.

4.1 The endoparasites of Bornean elephants

Trematodes, cestodes and nematodes were identified in this study: trematodes represented by *Fasciola*, cestodes represented by *Anoplocephala* and nematodes by a variety of strongyles.

*Fasciola*

*Fasciola* are classified as trematodes and are commonly known as liver flukes because adults inhabit the bile ducts of the definitive host.
*Fasciola* eggs are oval shaped with a distinctive golden yellow hue and operculum, a small lid, at one end (figure 2). The *Fasciola* eggs identified in this study (*n*=342) measured 120 to 140μm by 70 to 90μm.

**Figure 2 Fasciola egg.** Distinguishing features include its large oval shape, operculum and golden yellow hue.

**Anoplocephala**

*Anoplocephala* are classified as cestodes and are commonly known as tapeworms due to the flat tape-like appearance of the adult worm.

The morphology of *Anoplocephala* eggs is unique. They are nearly spherical with one or more flattened side(s), a thin smooth multilayered wall and a hexacanth embryo surrounded by a piriform apparatus (figure 3). *Anoplocephala* eggs identified in this study (*n*=150) measured approximately 60μm in diameter.

**Figure 3. Anoplocephala egg.** Distinguishing features include its nearly spherical shape with flattened side(s), a hexacanth embryo and piriform apparatus.
**Strongyles**

Strongyles are classified as nematodes and are a diverse group including hookworms of the stomach, intestines and caecum. Strongyle eggs have a typical ovoid appearance with thin, smooth walls surrounding a variable number of blastomeres (figure 4). They vary in size depending on species. Strongyles found during this study ($n=101$) varied from 60 to 70µm in length and 30 to 40µm in width.

Discriminant analysis of strongyle egg morphometry ($n=101$) reveals that strongyle egg measurements appear to be aggregated in a single cluster (see figure 5). Mean strongyle egg length was 67.64µm, SEM = 0.57, range = 54.10 to 84.18µm. Mean strongyle width was 38µm, SEM = 0.36, range 31 to 55µm.

**Figure 4. Strongyle egg.** Distinguishing features include its oval shape and thin, smooth walls surrounding blastomeres.

**Figure 5. Discriminant analysis of strongyle egg morphometry** Length and width of strongyle eggs are plotted to estimate how many different types of strongyles are present.
4.2 Parasite prevalence

Endoparasites were found to be ubiquitous in wild Bornean elephants with all samples yielding at least one type of endoparasite. Overall, *Fasciola* was found to be the most prevalent endoparasite with 70.2% (73/104) testing positive. The prevalence of strongyles 66.3% (69/104) was also very high and *Anoplocephala* tapeworm were common with half (52/104) of the samples testing positive (table 1).

In the Kinabatangan, the most prevalent endoparasites were strongyles 82.7% (43/52). *Anoplocephala* 69.2% (36/52) and *Fasciola* flukes 55.7% (29/52) were also prevalent.

In Tabin, the most prevalent endoparasites were *Fasciola* 84.6% (44/52). Strongyles 50.0% (26/52) and *Anoplocephala* 30.8% (16/52) were also found to be common.

<table>
<thead>
<tr>
<th>Parasite type</th>
<th>Fragmented habitat</th>
<th>Continuous habitat</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Fasciola</em></td>
<td>55.7% (29/52)</td>
<td>84.6% (44/52)</td>
<td>70.2% (73/104)</td>
</tr>
<tr>
<td><em>Anoplocephala</em></td>
<td>69.2% (36/52)</td>
<td>30.8% (16/52)</td>
<td>50.0% (52/104)</td>
</tr>
<tr>
<td>Strongyles</td>
<td>82.7% (43/52)</td>
<td>50.0% (26/52)</td>
<td>66.3% (69/104)</td>
</tr>
</tbody>
</table>

Prevalence of all types of endoparasites was significantly different between sites. The prevalence of *Fasciola* ($X^2 = 10.34$, df = 102, $p < 0.05$) was significantly higher in continuous compared to fragmented habitat. Conversely, strongyles ($X^2 = 4.98$, df = 102, $p < 0.05$) and *Anoplocephala* were significantly higher in fragmented than continuous habitat ($X^2 = 15.38$, df = 102, $p < 0.05$) (figure 6).
A survey of endoparasites in Bornean elephants

Figure 6. Parasite prevalence
Parasite prevalence of *Fasciola*, *Anoplocephala* and strongyle nematodes differ significantly between fragmented (LKWS) and continuous habitat (TWR).

4.3 Parasite load

There was no significant difference in the total parasite load between fragmented (343.4) and continuous habitat (356.9) \((W=1585, p>0.05)\). However, there were differences in respect to the load of particular parasite types (figure 7) and these concurred with differences in prevalence.

Figure 7. Parasite load in fragmented vs continuous habitat
Parasite load of *Fasciola*, *Anoplocephala* and strongyle nematodes differ significantly between fragmented (LKWS) and continuous habitat (TWR).
The mean load of *Fasciola* was significantly higher in continuous (238.0 epg) than in fragmented habitat (86.2) \((W=858.5, p<0.05)\). Conversely, the mean load of strongyles was significantly higher in fragmented (155.4) than in continuous habitat (81.3) \((W=1873, p<0.05)\). Likewise, the mean load of *Anoplocephala* was significantly higher in fragmented (101.8) compared to continuous habitat (37.6) \((W=1928.5, p<0.05)\) (table 2).

<table>
<thead>
<tr>
<th>Parasite types</th>
<th>Fragmented habitat</th>
<th>Continuous habitat</th>
<th>Mean load</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Fasciola</em></td>
<td>86.2</td>
<td>238.0</td>
<td>162.1</td>
</tr>
<tr>
<td><em>Anoplocephala</em></td>
<td>101.8</td>
<td>37.6</td>
<td>69.7</td>
</tr>
<tr>
<td><em>Strongyles</em></td>
<td>155.4</td>
<td>81.3</td>
<td>118.4</td>
</tr>
<tr>
<td>Mean load</td>
<td>343.4</td>
<td>356.9</td>
<td>350.2</td>
</tr>
</tbody>
</table>

Values for total parasite load were positively skewed overall (skew = 2.95) (figure 8) but more so in Tabin (skew = 2.74) than Kinabatangan (skew = 1.46) (figure 9).

**Figure 8. Positive skew of total parasite load (Skew=2.95)** Including all 104 samples from Kinabatangan and Tabin, parasite load data is positively skewed indicating parasite aggregation. Parasite load in a population is aggregated with many hosts harbouring few parasites and few hosts harbouring many parasites.
A survey of endoparasites in Bornean elephants

Figure 9. Positive skew of total parasite load in fragmented compared to continuous habitat. The skew is greater in continuous (Tabin) compared to fragmented habitat (Kinabatangan).

A. Positive skew of total parasite load - Tabin (Skew=2.74)

B. Positive skew of total parasite load - Kinabatangan (skew=1.46)
4.4 Parasite diversity

Trematodes, cestodes and nematodes were present in both fragmented and continuous habitat and overall, across both sites, mixed infections (diversity score >1) were common. The majority of samples (65.4%, 68/104) yielded mixed infections (figure 10).

![Figure 10. Diversity score in fragmented (LKWS) compared to continuous habitat (TWR)](image)

A maximum score of 3 indicates the sample was positive for 3 phyla of endoparasites: trematodes, cestodes and nematodes.

Mixed infections were significantly more frequent in continuous (80.8%, 42/52) compared to fragmented habitat (50.0%, 26/52) ($X^2 = 10.83$, df=1, p<0.05) (table 3).

**Table 3. Parasite diversity in fragmented vs continuous habitat**

*Fasciola, Anoplocephala* and strongyles were found in fragmented and continuous habitat but mixed infections (diversity score >1) were significantly more frequent in continuous habitat.

<table>
<thead>
<tr>
<th>Habitat</th>
<th>Parasite diversity score</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fragmented</td>
<td>50.0% (26/52)</td>
<td>34.6%</td>
<td>15.4%</td>
<td></td>
</tr>
<tr>
<td>Continuous</td>
<td>19.2% (10/52)</td>
<td>48.1%</td>
<td>32.7%</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>34.6% (36/104)</td>
<td>41.3%</td>
<td>24.0%</td>
<td></td>
</tr>
</tbody>
</table>
4.5 Other comparisons

Parasite load and bolus diameter
Considering all 104 samples from fragmented and continuous habitat, bolus diameter ranged from 4 to 26cm with a mean of 14cm. Spearman’s rank correlation reveals there is no significant correlation between parasite load and bolus diameter (Spearman’s rho = 0.097, df = 101, p > 0.05) (see figure 11) and there was no significant difference between bolus diameter in Kinabatangan compared to Tabin (W = 1499.5, p > 0.05).

Parasite load and sediment weight
Considering all 104 samples, the weight of sediment obtained from faecal samples ranged from 0.25g to 2.30g with a mean of 1.04g. Spearman’s rank correlation revealed there was a significant negative correlation between parasite load and sediment weight (Spearman’s rho = 0.221, df = 102, p < 0.05) (see figure 12).

There was no significant difference between sediment weight in Kinabatangan compared to Tabin (W = 1244, p > 0.05).
Figure 12. Parasite load and weight of sediment There is a significant negative correlation between parasite load and sediment weight.

Timing of fixation

The average count of the seven samples fixed at time of collection was 332.6 epg versus 138.9 epg when the fixation of these same samples was delayed. This indicates that delayed fixation results in significantly reduced egg counts (W=41, p < 0.05) (figure 13). Only 41.8% of the eggs detected in samples fixed immediately could be detected if fixation was delayed.

Figure 13. Timing of fixation On average, delayed fixation significantly reduces egg counts.
Repeat sampling of a mature bull

It was noted that Elvis’ bolus size varied from 16.5cm on the first encounter to 24cm on the second. His parasite load varied from 137.7epg from the sample collected in April to 317.7epg in May. Parasite types also varied with *Anoplocephala* and strongyles detected in April and *Fasciola* and *Anoplocephala* found in May (see table 4).

<table>
<thead>
<tr>
<th></th>
<th><em>Fasciola</em></th>
<th><em>Anoplocephala</em></th>
<th>Strongyles</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>24 April</td>
<td>0</td>
<td>45.9</td>
<td>91.8</td>
<td>137.7</td>
</tr>
<tr>
<td>22 May</td>
<td>211.8</td>
<td>105.9</td>
<td>0</td>
<td>317.7</td>
</tr>
</tbody>
</table>
5. Discussion
This study has established baseline data and infection patterns of endoparasites in Bornean elephants, providing the basis for a holistic One Health approach to conservation of endangered species in the face of ongoing anthropogenic environmental change.

Endoparasites are ubiquitous in wild Bornean elephants. The types of parasites identified are consistent with catalogues in other subspecies of Asian elephant. Notably, the prevalence and load of these parasites varies significantly between highly fragmented habitat in Kinabatangan and continuous habitat in Tabin suggesting that ecosystem and animal health are linked and habitat fragmentation is associated with altered infection dynamics.

5.1 Endoparasite prevalence and load

The total parasite prevalence (100%) and mean total parasite load (350.2 epg) across both sample sites can be considered extraordinarily high and such a heavy parasite burden suggests that wild Bornean elephants are under extreme pressure. These results are markedly higher than previous studies on other Asian elephant subspecies (Watve, 1993; Vidya and Sukumar, 2002) and are more akin to counts in intensively managed domestic species (Krecek et al., 1994; Watson et al., 1996). In a survey of over 200 captive and wild Indian elephants, Watve reported a mean total of 8 epg in the wet season to 25 epg in the dry (Watve, 1993). Vidya and Sukumar report an even lower average count of 1.44 epg to 2.50 epg in the wet season to 4.64 epg to 5.45 epg in the dry season (Vidya and Sukumar, 2002). The disparity between parasite load in this study and previous studies may be due to true differences in parasite prevalence and a range of host, parasite and environment factors some of which may be associated with habitat loss and fragmentation such as small population size, high population density and low genetic diversity. Alternatively, the differences in results may be attributed to differences in method. The sedimentation-flotation technique used in previous studies is considered less sensitive for quantitative counts than the McMaster method used in the current study.
A positive skew of total parasite load data indicates that the bulk of the values lie to the left of the mean which is consistent with parasite aggregation, an accepted paradigm in parasite distribution (Gulland, 1995). It is widely acknowledged that in any given population there is parasite aggregation, also known as over-dispersal, a highly skewed distribution of parasites. A large number of individuals carry few parasites and few individuals carry large numbers of parasites (Shaw and Dobson, 1995; Watve and Sukumar, 1997; Wilson et al, 2002). The causes and implications of parasite aggregation are not completely understood but studies suggest that the infection process and habitat may be significant determinants of aggregation patterns (Shaw and Dobson 1995; Shaw et al., 1998). An inequality of skew may indicate that the proportion of susceptible hosts in fragmented habitat is greater than in continuous habitat. Theoretically this means that parasites are likely to be more important as a selection pressure and regulatory influence in fragmented compared to continuous habitat (Wilson et al., 2003).

At first glance, the lack of significant difference in the total parasite load of samples in Kinabatangan (343.4) and Tabin (356.95) (W=1585, p>0.05) appears to suggest that degree of habitat fragmentation does not influence parasite load. However, this is unlikely to be a satisfactory explanation given that there are significant differences in the load of particular parasite types.

Prevalence and parasite load are a function of host, parasite and environmental factors. Host factors may be numerous including age, gender, individual behaviour, diet and water consumption, daily faecal volume and frequency, reproductive status and overall health. Parasite factors are many and include gender of adult worms, number of adult worms and their fecundity. Significant environmental factors include humidity, rainfall, climate, temperature and the frequency and intensity of external stressors. Considering variations in life cycles and routes of transmission, the prevalence and load of different types of endoparasites can be affected by different host, parasite and environmental factors. Therefore, it is appropriate to discuss prevalence and load of each endoparasite type in turn.
**Fasciola**

In order to understand patterns of *Fasciola* prevalence and load, it is necessary to take into account their unique life cycle. The life cycle of *Fasciola* is complex and indirect, relying on aquatic lymnaeid snails as intermediate hosts. The presence of lymnaeid snails has previously been confirmed in Sabah (Mas-Coma *et al*., 2005). *Fasciola* eggs hatch in water releasing miracidia which swim, enter intermediate hosts and go through several life stages, eventually emerging from snails as metacercariae. Definitive hosts become infected by ingesting food or water harbouring encysted metacercariae (Fowler and Mikota, 2006).

The high overall prevalence of *Fasciola* found in elephants in this study 70.19% (73/104) may be a reflection of the environmental conditions in Sabah which can be seen to be ideal for metacercariae. Metacercariae can persist for up to eight months in moist conditions and fodder (Fowler and Mikota, 2006).

There are several possible explanations why prevalence of *Fasciola* is significantly higher in continuous 85% (44/52) compared to fragmented habitat 56% (29/52) ($X^2 = 10.34$, df = 102, p <0.05). Firstly, the prevalence of vector borne parasites is often frequency-dependent rather than density-dependent meaning it is more strongly influenced by the frequency of encounters between vector and definitive hosts rather than host density (Smith *et al*., 2008). Hence even if host density is low, as is the case for elephants in Tabin, vector borne parasites like *Fasciola* can achieve high prevalence. Secondly, the abundance of trematodes has been shown to correlate significantly with the abundance of definitive hosts (Byers *et al*., 2010; Fredensborg *et al*., 2006; Lebarbenchon *et al*., 2007). Higher prevalence of *Fasciola* in Tabin may simply be a reflection of a greater total number of elephants.

Trematode parasites typically achieve high densities in snails living in marshy compared to degraded reserves (Lafferty and Gerber, 2002) which could explain higher prevalence of *Fasciola* in Tabin compared to Kinabatangan. Conditions and availability of suitable habitat for lymnaeid snail are important factors influencing the occurrence of *Fasciola* (Urquhart *et al*., 1996). Conditions and habitat for lymnaeid snails may be better in Tabin.
than in Kinabatangan. Microclimate is known to differ between fragmented and continuous habitat (Saunders et al., 1991) and the microclimate in Tabin may be more suitable for the development and dispersal of Fasciola life stages.

The numbers and persistence of snails and metacercariae may be influenced by agricultural toxins such as those in pesticides and effluent. For example palm oil mill effluent is generally pH 4 to 5 due to organic acids produced in the fermentation process (Lorestani, 2006; Ma, 2000). Acids are presumably hazardous to lymnaeid snails which are known to prefer near neutral pH (Laursen et al., 1989). Particularly away from the reserve edges, exposure to pesticides and effluent may be lower in Tabin than in Kinabatangan resulting in higher numbers and greater persistence of lymnaeid snails and metacercariae in Tabin.

Overall, the average load of Fasciola (162.1epg) found in this study is far in excess of egg counts in other Asian elephant parasite studies. The mean load of 86.2epg in Kinabatangan is at least in the realm of the load reported by Caple: up to 83 epg in nine captive elephants in Peninsula Malaysia (Caple et al., 1978). However, Karki reported lower counts with a mean Fasciola egg count of 4.89epg in the morning, 2.47epg in the afternoon and 2.76epg in the evening in 20 captive elephants in Nepal (Karki, 2005). The mean load of Fasciola in Tabin (238.0epg) is remarkably high and unprecedented. The average load of Fasciola in the current study is likely to be many times higher than previous studies on captive elephants due to differences in environment and management. For example, the elephants in Caple’s study were “fed sugar cane, bread and banana leaves” and were only “allowed to graze in the jungle while on a chain leash” so they were less likely to ingest as many infective metacercariae as wild elephants in Kinabatangan or Tabin who exclusively graze in the jungle.

This study adds to a growing body of evidence which indicates that Fasciola spp are prevalent and present in high numbers in Asian elephants. Stremme et al. (2007) reported that trematodes were amongst the most frequently encountered endoparasites in their survey of 127 Sumatran elephants.
Clinical cases of acute and chronic fascioliasis in elephants have been reported (Fowler and Mikota, 2006). The health status of elephants sampled in the present study could not be accurately assessed but from brief external examination at a distance, none showed outward signs consistent with clinical fascioliasis despite high prevalence and load.

**Anoplocephala**

The *Anoplocephala* life cycle is indirect with oribatid mites as intermediate hosts. Five species of oribatid mites have been found to serve as intermediate hosts for *Anoplocephala* in elephants (McAloon, 2004). Definitive mammalian hosts become infected by inadvertently ingesting mites containing tapeworm larvae.

The overall high prevalence of *Anoplocephala* across both sites (50%, 52/104) is likely to be a reflection of elephants’ grazing behaviour and microenvironment. The majority of studies on the epidemiology of *Anoplocephala* come from equine literature where it has been suggested that high prevalence is associated with frequent grazing of permanent pastures for consecutive years and micro-environmental conditions, such as soil type and acidity, favouring oribatid mites (Ihler et al., 1995). These factors may explain the higher prevalence of *Anoplocephala* in Kinabatangan (69.2%, 36/52) compared to Tabin (30.8%, 16/52) ($X^2 = 15.38$, df = 102, p <0.05). The microenvironment in Kinabatangan may be more favourable to oribatid mites and elephants in Kinabatangan may be seen to be feeding at the same sites year upon year as habitat fragmentation precludes elephant movement to new feeding grounds.

There is scant literature on tapeworm in Asian elephant. It appears that the high overall prevalence of *Anoplocephala* (50%, 52/104) found in Bornean elephants is unprecedented. McAloon (1997) found 33% of captive Indian elephants were positive for *Anoplocephala*. Other studies have found a much lower prevalence of cestodes. Vidya and Sukumar (2002) report a prevalence of just 0.6% in wild elephants and Chandrasekharan et al. (2009) reported only 12 cases of *Anoplocephala* in thirty years of screening captive and wild elephants in Kerala, India. Inconsistencies between the current and previous studies may be due to variations in host, parasite or environmental factors or differences in methods.
and management. For example, some of the captive elephants included in the study by Chandrasekharan et al. received veterinary treatment.

As there are no reports of *Anoplocephala* egg counts readily available in the literature, it is difficult to assess whether a mean load of 101.8 epg in Kinabatangan, 37.6 epg in Tabin and 69.7 epg overall is particularly high or low. Nevertheless, the results of the current study indicate that the load of cestodes is significantly higher in Kinabatangan compared to Tabin and this may be attributed to the effects of habitat loss and fragmentation on infection dynamics.

**Strongyles**

The life cycle of elephant strongyles are not well characterised but is assumed to be direct as are strongyles in other host species. L4, L5 and adult strongyles, depending on species, reside in the stomach, caecum or intestines. Transmission is by faecal-oral spread. Eggs are passed in faeces and under warm humid conditions such as in Sabah, hatching would be expected within one to two days. Larvae moult through two life stages in the faeces to become infective L3 in four to six days. L3 crawl onto vegetation and are consumed by the host.

The overall high prevalence of strongyles (66.3%, 69/104) indicates that there is a high potential for direct faecal oral transmission of parasites and infectious disease in Bornean elephants in continuous and fragmented habitat. This is particularly concerning as parasites and disease transmitted by close contact have been shown to be more likely to cause increased extinction risk than those transmitted by other routes (Pedersen et al., 2007).

The higher population density in fragmented compared to continuous habitat (2.15 compared to 0.6 elephants per km$^2$) is a plausible explanation for the significantly higher prevalence ($X^2 = 4.98$, df = 102, p<0.05) and load (W=1873, p <0.05) of strongyles in fragmented habitat. This alone could explain higher prevalence and load of strongyles in fragmented habitat as the level of exposure to strongyle eggs in the environment increases with increased population density. High definitive host population density facilitates the
success of parasite reproduction and increases faecal oral transmission (Anderson and May, 1979). Extensive research in numerous taxa has established that host density is a key factor contributing to prevalence, load and diversity of nematodes (Wiegertjes and Flik, 2004 in avians; Takemoto, 2005 in fish; Sutherland and Scott, 2009 in sheep and cattle; Suzan et al, 2012; Trejo-Macias and Estrada, in press in primates).

The most likely alternative explanations for the higher strongyle prevalence in fragmented compared to continuous habitat are physiological and or nutritional stress. Chronic stress in animals, such as that associated with human contact, is known to predispose to parasites and disease (Schwitzer et al, 2010). Lack of suitable food and deficiencies in dietary components, particularly protein and energy, also influence susceptibility to nematodes (Chapman et al., 2006). Studies in free living red colobus monkeys indicate that food availability has a strong influence on a number of parasite indices and supports the suggestion that nutritional status affects host immunity to parasites (Gillespie et al., 2005; Gillespie and Chapman, 2006; Gillespie and Chapman, 2008). This is supported by extreme case studies in African elephant in Kenya where dietary stress and parasitism had a synergistic effect leading to mass mortalities (Obanda, 2011). These explanations can still be linked to habitat loss and fragmentation. Elephants in fragmented habitat may be seen to have more frequent human contact and also limited access to suitable feeding grounds than elephants in continuous habitat and therefore may be under greater physiological and dietary stress resulting in higher susceptibility to nematodes.

Higher strongyle prevalence in fragmented compared to continuous habitat is also likely be associated with exposure to immunotoxins such as certain agricultural waste products, herbicides, pesticides and accumulated heavy metals, which can be immunosuppressive given chronic or frequent exposure. Theoretically, immunotoxin exposure is more likely in fragmented habitat in closer proximity to plantations and human settlements than in continuous habitat. It is estimated that 53 million tonnes of palm oil effluent is produced in Malaysia every year (Lorestani, 2006). Exposure to effluent and toxins may directly and indirectly predispose to disease and parasitises (Lloyd, 1995).
Differences between parasite variables in Kinabatangan and Tabin may be associated with differences in individual and host population immunity. However, there is no baseline immunological data available in Bornean elephants and to establish this would require invasive and impractical testing. Indeed, studies on immune regulation and helminth parasites in free living wildlife are rare (Schad et al., 2005).

Strongyle nematodes are perhaps the most widely reported genera of endoparasites in Asian elephants. Previous studies have also found a high prevalence of strongyles. Watve (1993) reported a prevalence of 87 to 98% in 207 captive and wild Indian elephants over three seasons and Vidya and Sukumar (2002) found a prevalence of 84% in 320 free ranging wild Indian elephants in Mudumulai Wildlife Sanctuary where population density of 2.95 elephants per kilometre square is even higher than in Kinabatangan. Studies of strongyles in captive elephants have also found a high prevalence. Chandrasekharan et al. (1995) reported a strongyle prevalence of 91.27% in captive Indian elephants in Kerala.

The results of the present study strongly suggest that habitat fragmentation is associated with an increased prevalence of strongyle nematode infection. Likewise, the prevalence of parasites including nematodes in red colobus and mangabey in Kenya (Mbora and McPeek, 2009) and howler monkeys in Mexico is higher in fragmented than continuous habitat (Trejo-Macias et al, 2007).

The significant differences in parasite prevalence and load between fragmented and continuous habitat indicate that endoparasites are an important tool in wildlife conservation and can be used as an indicator of ecosystem and animal health. The hypothesis that endoparasites are more prevalent and present in greater numbers in fragmented compared to continuous habitat has been shown to be an overgeneralisation. The results of the present study indicate that generalisations across parasite types should be avoided and analysis should consider the complexities of parasite life cycles and range of host, parasite and environmental factors influencing the infection dynamics of different types of endoparasites.
5.2 Parasite diversity

The high frequency of mixed infections overall (65.4%, 68/104) suggests that Bornean elephants are susceptible to a myriad of endoparasites and that environmental conditions across Sabah are conducive to the survival and transmission of a variety of parasites.

Higher frequency of mixed infections in Tabin (80.8%, 42/52) compared to Kinabatangan (50.0%; 26/52) \((X^2 = 10.83, \text{df}=1, p<0.05)\) may be a reflection of overall biodiversity in continuous versus fragmented habitat. Evidence indicates that smaller, disconnected patches have lower species diversity than continuous habitat (MacArthur and Wilson, 1967; Robinson and Quinn, 1992; Fahrig, 2003).

Alternative explanations for a higher frequency of mixed infections in continuous versus fragmented habitat include animal movement and grazing behaviour. Meta-analysis in primates has suggested that greater freedom of animal movement can result in feeding at a greater variety of locations and on more different types of fodder thus increasing exposure to a greater variety of endoparasites (Nunn et al, 2003). Elephants in Tabin have greater freedom to move to different feeding grounds and consequently may have greater exposure to a variety of endoparasites.

The results of this study appear to be inconsistent with previous studies on parasite diversity in elephants. The incidence of mixed infections in Tabin is higher than previous estimates of mixed endoparasite infection prevalence in Asian elephants. Only 22.5% of 40 captive temple elephants surveyed by Jani harboured mixed infections (Jani, 2008) and Vidya and Sukumar found mixed infections in just 11.9% of samples from wild Indian elephants (Vidya and Sukumar, 2002). The higher frequency of mixed parasite infections in the present study may be associated with differences in study methods or sites. Parasite diversity is said to be higher in tropical areas (Bush et al., 2001) and the present study was conducted in a biodiversity hotspot. Parasite diversity may simply be a reflection of the biodiversity in the study sites (Hechinger and Lafferty, 2005). Alternatively, the methods used in previous studies may not have had the power to detect the same diversity as the McMaster method used in the present study.
This study has found a higher frequency of mixed infections in continuous compared to fragmented habitat which appears to contradict previous studies on parasite diversity and habitat disturbance. Maciag (2010) found parasite diversity was higher in lemurs in degraded compared to primary forest. Likewise, Junge et al. (2011) found indri in disturbed forest have a higher diversity of parasites compared to those in undisturbed forest. However, it is difficult to compare studies which vary in host and parasite species, habitat type, matrix composition, fragment size, duration of study and methodology. Most notably, as highlighted by Suzan et al. (2012) previous studies have invariably only looked for closely related parasites whereas the present study includes three different phyla: trematodes, cestodes and nematodes. Hence the definition of ‘mixed’ differs between the present and past studies. ‘Mixed’ in the present study meant more than one phyla whereas Maciag (201) took ‘mixed’ to mean more than one species of oxyurid nematode. Consequently, comparisons and generalisations about the relationship between habitat fragmentation and parasite diversity must be made with caution.

In an attempt to investigate the diversity of strongyle species in Bornean elephants, discriminate analysis of strongyle egg morphometry was undertaken to differentiate between eggs of different species of strongyles. At first glance, the single cluster of data points on discriminant analysis appears to suggest only one species of strongyle (see figure 5). However, this is unlikely to be a satisfactory explanation given overlapping measurements and wide range of lengths 54.10 to 84.18μm and widths 31 to 55μm. More than likely, the strongyle egg morphometric data collected in this study indicate that Bornean elephants harbour a variety of strongyle species including Murshidia and Quilonia. Mean egg length of 67.64μm, SEM = 0.57 and width 38μm, SEM = 0.36 would fit the morphometrics of Murshidia or Quilonia, commonly reported genus of Asian elephants (Fowler and Mikota, 2005).
5.3 Other comparisons

Parasite load and bolus diameter
The lack of correlation between parasite load and bolus diameter (Spearman’s rho = 0.097, df=1, p>0.05) may indicate that there is no relationship between age and parasite load in Bornean elephants. This would concur with Watve’s results which demonstrated that there is no correlation between parasite load and host age in Indian elephants (Watve, 1992). Alternatively, it may imply that bolus diameter is not a reliable proxy for age in wild Bornean elephants. This hypothesis is supported by bolus measurements recorded for ‘Elvis’ which suggest that there is intra-individual variation in bolus diameter likely associated with diet and water intake influencing faecal consistency. Such variation would indicate that one off bolus measurement is an unreliable method of age estimation.

Parasite load and sediment weight
The significant negative correlation between parasite load and sediment weight (Spearman’s rho = 0.221, df = 102, p < 0.05) is likely a reflection of diminished visibility with increasing sediment weight resulting in lower egg counts.

Immediate versus delayed fixation
Immediate fixation of samples is important for accurate faecal egg count as delayed fixation was found to result in a significantly lower egg count (W=41, p<0.05). This not only highlights the merits of the sample collection protocol used in this study but also indicates the importance of collecting freshly deposited samples. A delay of approximately twelve hours between collection and fixation significantly reduces the egg count. This is likely to be due to eggs developing and hatching.

Repeat sampling of a mature bull
Repeat sampling of the mature bull identified as ‘Elvis’ indicates that there is intra-individual variation in bolus size, parasite load and diversity suggesting that estimates of these variables taken at one point in time may not reflect measures over time.
5.4 Limitations

It must be noted that there are several limitations to this study including the scale, inability to account for the myriad of confounders, the possibility of false positive and false negative results and potential duplication of samples.

Given the limited sample size and relatively small temporal and spatial scale of this study, generalisations about host parasite environment interactions across time and space are drawn with caution. Generalisations about infection dynamics on a population and habitat wide scale would require long-term studies of wild elephants across their range.

A myriad of host, parasite and environmental variables which affect parasite prevalence, load and diversity could not be accounted for in this study. Indeed, many confounding host variables are extremely difficult nye impossible to measure accurately and comprehensively over time and space in wild free-ranging elephants including overall state of health, concomitant disease, nutrition and immunocompetence as these would require repeated invasive procedures to assess.

The McMaster method, similarly to any diagnostic test, entails the possibility of false negative and false positive results. False negatives can result from burdens below the minimum 10 epg (the detection threshold for the special modification of the McMaster method), infection with a single sex of parasite or immature larval stages, low parasite fecundity, variable daily egg output, fluctuating faecal output and variations in rate of passage. For example, detection of Fasciola can be unpredictable because eggs are expelled intermittently depending on evacuation of the gall bladder (Briskey 1998). False negatives may also have arisen because particularly heavy eggs are less likely to show up on flotation methods for example eggs of Toxocara roundworms. False positives may result from errors of identification though results were verified by experts (Fox, personal communication).

A significant limitation of this study is duplication, the repeated collection of faecal samples from the same individuals. Due to terrain, visibility, practical limitations, safety
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concerns and time constraints, it was unfortunately not possible to approach elephants closely to identify all individuals sampled or establish demographics such as age, sex or body condition scores as performed in large scale, long term parasite surveys of elephants in more open habitat (Vidya and Sukumar, 2002). In this study, groups were observed prior to collection and boluses of different sizes were sampled to minimise duplication. However, it is still possible that individuals were inadvertently sampled more than once particularly in Kinabatangan where samples were collected during separate expeditions weeks apart, ample time for elephants to move from one area to another.

5.5 Future research

This study has developed an effective, practical and repeatable protocol to screen free ranging Bornean elephants for endoparasites. This opens many avenues for further research. The spatial and temporal scale of the project could be expanded, the methods refined, novel population surveys conducted, scope of the study widened and findings can be incorporated into conservation management.

Expanding the scale
A long term investigation into elephant endoparasites across Sabah is needed to further investigate the patterns identified in the present study and examine confounders and interacting threats. A long term survey across Sabah could overcome the problem of duplication by providing the opportunity to selectively sample only elephants directly observed defaecating. This would also allow for collection of demographic data including sex and estimation of age as well as important factors such as body condition and reproductive status. While, in the case of other study species, it may be advantageous to carry out genetic identification of faecal samples, the same might not be said for Bornean elephants. It is suspected that they lack the genetic diversity necessary for such methods of identification using currently available markers (Othman, 2012).

Expanding the temporal and spatial scale of this study would allow closer examination of links between infection dynamics and stressors associated with habitat loss and fragmentation. For example, data from radio collared elephants could be incorporated in
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more sophisticated models to establish the frequency and proximity of elephants to human settlements and plantations in the weeks preceding sample collection to assess how human wildlife contact affects animal health.

This study provides the baseline data and methodology necessary for future investigations. For example, to further investigate links between habitat fragmentation and disease it would be advantageous to compare parasite prevalence, load and diversity between elephants who have spent weeks to months in: i) Primary forest, ii) Secondary forest, iii) Plantations. This may be possible if access to primary forest improves in the future. Screening the captive Bornean elephants at Lok Kawi Wildlife Park, Kota Kinabalu would also be beneficial as this would allow for analysis based on a variety of known variables, including age, sex, nutrition, activity patterns, medical history, climate and population density.

**Refining the methods**
There is much room for refinement of methods used in this study particularly in terms of parasite identification. Other more accurate and sensitive faecal egg counting methods are available but would require more time, training and equipment to carry out in the field. For example parasites could be identified not just by egg morphology and morphometry but also by larval identification either by hatching eggs collected from faeces or from mature female parasites collected from deceased elephants on necropsy. A greater sample size and advanced modelling such as K-means clustering could be performed on egg dimensions to categorise strongyle eggs into different operational taxonomic units (Vidya and Sukumar, 2002). Even more sophisticated methods may also soon be available with the very recent development of the first DNA markers to identify strongyle species in African elephants (McLean et al., 2012). More accurate endoparasite species identification would refine the scale and increase precision to better define differences in parasite diversity between fragmented and continuous habitat.

**Conducting a novel elephant population survey**
The confirmed presence of *Fasciola* in Bornean elephants provides the interesting opportunity to apply recently developed population abundance survey methods. On the
basis that the abundance of parasites is closely linked to the abundance of suitable hosts, trematode surveys have been shown to be reliable predictors of abundance of cryptic host species (Fredensborg et al., 2006; Byers et al., 2010). Hence it may be possible to pilot population surveys of elephant via surveys of non-adult phases of trematodes in lymnaeid snails.

**Widening the scope of the study**

Methods used in this study could be employed in future One Health research. For example endoparasite surveys could be combined with measures of faecal cortisol and reproductive hormones. Correlating faecal egg counts and parasite prevalence, load and diversity with these parameters would provide further insights into links between ecosystem and animal health by exploring the relationship between habitat loss, fragmentation, infection dynamics, stress physiology and reproduction.

There remains much to learn about interacting threats and their impact on endangered species and it is critical that future research explores the links between ecosystem and animal health. Now and in the future, effective endangered species conservation requires an understanding of the interactions between anthropogenic threats. Habitat loss and fragmentation occur in parallel to other significant threats which can influence infection dynamics for example climate change. Recent modelling has demonstrated that global climate change is likely to make it easier for parasites to infect hosts and could exacerbate the effects of some diseases (Raffel et al., 2012). The baseline data collected in this study and methodology could be used to investigate the synergistic effects of habitat loss, fragmentation, climate change and other threatening processes on infection dynamics.

**Incorporating findings into conservation management**

Results of this study indicate that infection patterns vary with habitat loss and fragmentation and this can be incorporated into conservation management, lobbying, decision making and monitoring particularly given potential animal health implications of continuing plantation activities versus projects such as the Bornean Conservation Trust Green Corridor Plan which aims to increase habitat connectivity in Sabah (BCT, 2011). Findings of this study provide further support for habitat preservation and restoration and
illustrate how endoparasite surveys can be employed in conservation. Considering that endoparasites occur in virtually all ecosystems, the protocols and techniques used in this study can also be applied to assess the health and status of other endangered species under threat from anthropogenic habitat disturbance.

5.6 Concluding comments

Practical methods have been developed to gather the first baseline data on endoparasites in wild Bornean elephants. Endoparasites have been found to be ubiquitous in elephants surveyed and the prevalence and load are generally high compared to existing reports on endoparasites in Asian elephants suggesting that ecosystem and animal health are under particular pressure in Sabah, Malaysia, a biodiversity hotspot under threat.

Prevalence, load and diversity vary significantly between fragmented and continuous habitat, supporting the hypothesis that habitat loss and fragmentation can affect infection dynamics. The frequency of mixed infections is significantly higher in continuous habitat possibly due to higher biodiversity or greater freedom of animal movement in continuous compared to fragmented habitat. Trematodes are more prevalent and the average load is significantly higher in continuous habitat which may reflect the larger total population of elephants or more favourable conditions for intermediate hosts. Cestodes are more prevalent and the average load is significantly higher in fragmented habitat which may reflect limited freedom for elephants to move to new feeding grounds. Most notably, the prevalence and load of nematodes was found to be significantly higher in fragmented compared to continuous habitat indicating that endangered elephants in fragmented habitat are at increased risk of disease spread by direct faecal-oral transmission.

This is a step towards a One Health approach to elephant conservation. The demonstrable links between ecosystem and animal health have significant implications for conservation management which must consider interacting threats to protect endangered species in situ. Given these links, this study provides evidence that endoparasites can serve as novel tools to assess and monitor the status of wildlife threatened by habitat loss and fragmentation.

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Appendix

A. Elephant faecal sample collection protocol (Modry, personal communication)

**Equipment**
- 25ml polypropylene specimen containers
- 95% ethanol
- Parafilm sealing tape
- Permanent marker pens
- Measuring tape
- Surgical gloves

1. Pre-prepare 25ml specimen tubes approximately half filled with ethanol (no need to measure exactly just ensure the faecal sample is submerged in ethanol)
2. With a gloved hand, take samples from the centre of several different boluses in the dung and place in pre-prepared tubes. Ensure faeces are covered in ethanol. May need to top up.
3. Label each tube with sample number (elephants were very close by during collection so other data such as date of collection were recorded post-collection)

B. Preparation of samples (Fox, personal communication)

1. Pour entire faecal sample into a mesh sieve sitting over a beaker
2. Manually agitate gently for 30 seconds (do not squeeze)
3. Pour contents of beaker into specimen containers and centrifuge 1500rpm for 2 minutes
4. Pour off ethanol
5. Fill specimen container with tapwater, resuspend sediment by gently inverting and then centrifuge again 1500rpm for 2 minutes
6. Pour off tapwater

C. McMaster method (special modification) (MAFF, 1986)

The sensitivity of the Modified McMaster method may be increased from 50 epg to 10 epg by incorporating the following simple modifications:
1. Weigh 4.5g of faecal sediment (or record weight \(x\) if \(<4.5g\))
2. Add saturated salt solution to make a total volume of 45ml (sodium chloride NaCl was used consisting of 350g of salt added to 1000ml of tap water with an estimated spg of 1.33)
3. Count the parasite eggs present over the entire area of each chamber (not just within grids)
4. Multiply the total number of eggs by 10 to give epg
5. If a sample of \(<4.5g\) was used multiply the faecal egg count (obtained in (4) above) by \(4.5/x\) where \(x\) is the weight of the faecal sediment.
### D. Excerpt from Excel spreadsheet of results

<table>
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<th>Sample no.</th>
<th>Latitude</th>
<th>Longitude</th>
<th>Bolus diameter(cm)</th>
<th>Sediment weight (g)</th>
<th>Fasciola EPG</th>
<th>Anoplocephala epg</th>
<th>Strongyle epg</th>
<th>Total epg</th>
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Appendix E. Example of code used in R

i. R script used and read out obtained for Mann Whitney U test for strongyle load in Kinabatangan versus Tabin

```r
> wilcox.test(strongyle$LKWS,strongyle$TWR)

Wilcoxon rank sum test with continuity correction

data:  strongyle$LKWS and strongyle$TWR
W = 1873.5, p-value = 0.0005409
alternative hypothesis: true location shift is not equal to 0
```
“All is connected. No one thing can change by itself.”
- Paul Hawken, author and environmentalist