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The Ecology of Gastrointestinal Parasites in a Population of *Alouatta pigra* In Southern Belize

by

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Abstract

This study investigated the relationship between parasites, diet and behaviour and assessed differences in parasite prevalence by group, age class, sex, season and the amount of human contact in a population of black howler monkeys (*Alouatta pigra*) in Southern Belize. Four parasites were recovered (*Giardia duodenalis* [Assemblage B], *Trichostrongylid* sp., *Trichuris* sp., and *Controrchis* sp). As *Controrchis* sp. requires an arthropod host for transmission and *A. pigra* are folivorous and frugivorous, I investigated the source of *Controrchis* sp. as ants ingested when feeding on *Cecropia peltata*, a tree with a mutualistic relationship with *Azteca* sp. ants. As predicted, there was a significant positive relationship between the time spent feeding on *C. peltata* and the individual abundance of *Controrchis* sp. As pioneer tree species such as *C. peltata* are the first to grow in disturbed forests, these results suggest a direct link between habitat disturbance and parasitism.

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Dedication

I would like to dedicate this thesis to my mother Katharine, for she has instilled in me the strength and determination necessary to take on and complete this project. Her unwavering support and faith in my abilities has allowed me to cope with difficult circumstances both in the field and the laboratory. When my belief in myself has waned she has always been there to reinforce my confidence and remind me to put things in perspective. Though this entire process she has been my biggest supporter and it is to her I owe the perseverance that has allowed me to complete this project; thanks Mom, I love you!!!

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CHAPTER 1- INTRODUCTION

Parasites as a Selective Force

Over the past two decades the majority of primatological theory has focused on the evolutionary forces that are responsible for shaping primate behavioural patterns and social systems; the way in which ecological factors such as the food supply or the risk of predation affect primate behaviour are well understood (vanSchaik, 1983; 1989; Sterck et al. 1997; Clutton-Brock and Harvey, 1977; Chapman et al. 2009). Although parasites fill an important niche in natural ecosystems and virtually all animals are infected with at least one species of parasitic organism (Price, 1980), the impact of parasites as a selective force shaping primate behaviour has been largely ignored. Parasites evolve strategies to increase their infection and transmission efficiency (i.e. antibiotic resistant bacteria), and primate species may develop behavioural strategies to combat parasite infections.

Numerous species of ape have been documented to combat gastrointestinal parasite infections through medicinal plant use and it has been speculated that other species of primates may reduce exposure to parasites through selective sleeping grove use or defecation from low in the canopy (Huffman, 1997; Hausfater and Meade, 1982; Gilbert, 1997). Just as host behaviour represents a constant selective force for parasites in the wild, recent studies suggest that parasites represent a selective force that may shape the behaviour of primates. In a wild environment, faced with the constant threat of parasitosis (infection with a parasite), behaviors aimed at the control or prevention of endoparasitic infections may be critical to the survival of individuals. Primates have faced exposure to disease for millions of years, and animals that survive to pass on their

genes may be able to do so, in part, because they are better equipped to tolerate environments where the potential for disease exists.

The long life spans of primates, in conjunction with a high degree of contact among group members may result in many opportunities for contact with parasites (Nunn and Altizer, 2006). In addition, the phylogenetic closeness shared by human and non-human primates means the potential for cross-species disease transmission exists and represents an increasing threat in areas where multiple primate species live sympatrically. Gastrointestinal parasitosis may cause a number of pathologies, and the impact of this type of infection on diet and behavior has been documented among great ape populations (Huffman, 1997). Emerging evidence suggests that specific behavioral patterns displayed by certain primate species may be adaptive strategies aimed at the control of endoparasitism (Hart, 1990; Huffman, 1997; Nunn and Altizer, 2006).

Alouatta pigra (Belizean black howler monkeys) are classified as an endangered New World primate species, and are endemic to regions of Belize, Mexico and Guatemala (IUCN, 2008; Nowak, 2000). The survival of many *A. pigra* populations is threatened by factors such as the expansion of residential and commercial development, agriculture and aquaculture, all of which have the potential to dramatically alter the physical environment (Stoner and Di Pierro, 2006; IUCN, 2008). Exposure to novel environments may affect parasitic infections in many different ways including: introducing new pathogens to primate populations, changing the available food resources, and decreasing immune system function (through increasing cortisol levels).

While endoparasitism has been thoroughly studied in populations of *A. belzebul* (red-handed howler monkey), *A. caraya* (black and gold howler monkey) and *A. palliata*

(mantled howler monkey) (Stuart et al. 1998), there are few studies of parasitism in *A. pigra* (Vitazkova and Wade, 2006; Stoner and Gonzalez, 2006; Eckert et al. 2006; Trejo-Macias et al. 2007). In October of 2001, the population of *A. pigra* in Monkey River was in the direct path of a category four hurricane that resulted in complete defoliation of the forest and reduced the monkey population by 40% (Behie and Pavelka, 2005). As parasitosis may be especially detrimental to the health of individuals experiencing nutritional or habitat-specific stresses, it is necessary to determine the intrinsic host characteristics and environmental parameters that influence parasitic infection in this population of *A. pigra*.

Research Objectives

The objectives of this study were to document the intestinal parasites present in the Monkey River population of *A. pigra*, and to identify how endoparasitic infections relate to behaviour, activity, diet, and certain features of the environment. This research explored variation in parasite prevalence among age classes, sexes and groups, and assessed behavioural and dietary correlates of parasitism. The route of *Controrchis* sp. infection was investigated, as were the relationships between seasonality and parasitism and contact with humans and parasitism. In conjunction with data on levels of parasitosis, behavioural data was used to investigate if selective defecation from low in the canopy serves as a possible parasite avoidance strategy.

CHAPTER TWO: LITERATURE REVIEW

Parasitic Infection in Primates

Primates are the definitive hosts in the lifecycles of a number of parasite species, and many epidemics caused by microparasites such as Yellow Fever and Ebola have had devastating impacts upon primate populations (Walsh et al. 2003b). This study focused on macroparasite and *Giardia* sp. infections in a population of *A. pigra* in Belize. A number of intestinal macroparasites have been recovered from *A. pigra* in Central and South America (Table 1), yet to date there are only two studies that report the endoparasites of Belizean *A. pigra* (Eckert et al. 2006; Vitazkova and Wade, 2006).

Table 1: Endoparasites of *Alouatta pigra*

Parasite	Source	Recovered in Monkey River
Platyhelminthes		
Trematode (unkn)	Trejo-Macias et al. 2007	
Controrchis biliophilus	Trejo-Macias et al. 2007 Vitazkova and Wade, 2006	YES
Digenean (unkn)	Stoner and Gonzalez, 2006	
	Eckert et al. 2006	
Nematodes		
Trypanoxyuris sp.	Trejo-Macias et al. 2007 Vitazkova and Wade, 2006	
Ascarid	Eckert et al. 2006	
Strongylid sp. (unkn)	Trejo-Macias et al. 2007 Eckert et al. 2006 Stoner and Gonzalez, 2006	
Trichostrongyloides sp.	Stoner and Gonzalez, 2006	YES
Entrobius sp.	Stoner and Gonzalez, 2006	
Oxyurid	Eckert et al. 2006	
Trichuris sp.	N / A	YES
Protozoa		
Blastocystis sp.	Stoner and Gonzalez, 2006	
Giardia sp.	Vitazkova and Wade, 2006	YES
Entamoeba sp.	Stoner and Gonzalez, 2006 Eckert et al. 2006 Vitazkova and Wade, 2006	
E. coli	Stoner and Gonzalez, 2006	
Isospora sp.	Stoner and Gonzalez, 2006	
Iodamoeba butschlii	Eckert et al. 2006	

Parasitic organisms are typically classified as either macroparasites or microparasites (Anderson and May, 1991). Microparasites include viruses, bacteria, fungi and protozoa, while helminths (worms) and arthropods (invertebrates) are classified as macroparasites. Although an extremely diverse number of parasites are contained in these two groupings, there are several key distinctions between micro- and macropàrasites.

Microparasites are characterized by antigenic simplicity, typically reproduce asexually, multiply within the host, and have short generation times (relative to that of macroparasites). The persistence of microparasitic infections depends on the presence of infected hosts (i.e. prevalence) (Anderson and May, 1991). Whereas macroparasites tend to cause chronic, persistent infections in host populations, microparasitic infections are often linked with sudden epidemics in nature, resulting in the removal of susceptible hosts from the population via immunity or death (Gulland, 1995). The detrimental effect of microparasitic infection on the health of domestic animals has been well documented and infection with viruses such as rabies and malaria are known to cause acute illness and even death (Brack, 1987; Anderson and May, 1979; May and Anderson, 1979). Conversely, as macroparasites may cause few clinical symptoms in the host, the cost of these infections to the fitness of animals is typically more difficult to determine (Nunn and Altizer, 2006).

Macroparasites typically reproduce sexually, and multiplication occurs by the release of infective stages (eggs or larvae) into the environment. These parasites tend to have long generation times (relative to that of microparasites), and the persistence of disease depends on the number of parasites per host (i.e. abundance) (Anderson and May, 1991). The large physical size and antigenic complexity of macroparasites (compared to

microparasites) results in decreased efficacy of host immunity to macroparasitic infections, leaving hosts susceptible to continual re-infection.

Helminths (parasitic worms) are the parasites most commonly recovered from wild primate populations (Nunn et al. 2003a; Vitone et al. 2004). Helminths are a taxonomically diverse set of parasites and include organisms from the phyla Nematoda, Platyhelminthes (e.g. cestodes, digenean trematodes) and Acanthocephala (e.g. thorny headed worms). Helminth parasites can have direct or indirect lifecycles and use a diverse number of transmission modes in order to complete their lifecycles; certain helminthes require small inter-host distances for transmission, while others may be successfully transmitted over long distances (Anderson and May, 1991). Taken together, these facts suggest that there is no single strategy that confers minimal risk of exposure to *all* macroparasitic species to which a host is vulnerable.

Nematoda

Commonly known as roundworms, Nematoda is the most diverse phyla of helminth. Although lifecycles within the phylum are fairly uniform, there is diversity in the location of adults and larvae within the host (Roberts and Janovy, 2005). Nematodes also exhibit a variety of complex and direct modes of transmission. The developmental pattern typically includes the production of an egg, from which the larval (L1) or juvenile stage develops. The lifecycle of most nematode species includes four larval stages (L1, L2, L3, L4), separated by molts, before development into the adult stage in the final (definitive) host (Roberts and Janovy, 2005). In nematodes with direct lifecycles, it is usually the L1 or L2 that are infective to the definitive host. In nematodes with indirect

lifecycles, the L1 or L2 are commonly infective to the intermediate host (if present), and the L3 is infective to the definitive host.

Nematodes recovered from *Alouatta* sp. include: *Enterobius* sp. (Stoner and Di Pierro 2006), *Ascaris* sp. (Eckert et al. 2006; Stuart et al. 1990), *Trypanoxyuris* sp. (Stuart et al. 1990,1998; Vitazkova and Wade, 2007), *Trichuris* sp. (Phillips et al. 2004), unidentified species of *Strongylid* (Stuart et al. 1998; Eckert et al. 2006, Stoner and DiPierro, 2006) and *Trichostrongyloides* sp. (Stoner and DiPierro, 2006). Few species of nematodes are lethal, yet many can cause harmful pathogenesis in the host. Infection with *Ascaris lumbricoides* can cause inflammation (through larval migration), tissue damage (through aberrant migration), and malnutrition and intestinal obstruction (in the adult form) (Roberts and Janovy, 2005). Heavy infections (100+ worms) with *Trichuris* sp. in humans can lead to anemia (through blood loss), intestinal inflammation, dysentery, growth retardation and rectal prolapse (Ramdath et al. 1995; Cooper et al. 1992).

Cestoda

Commonly known as tapeworms, adult cestodes are found in the lumen of the digestive tract of vertebrates, while larval stages parasitize invertebrate or vertebrate intermediate hosts (Nunn and Altizer, 2006). All species of cestode require at least two and sometimes three hosts to complete their lifecycles and transmission occurs exclusively via the food chain. The most common sequence of life cycle involves the ingestion of eggs deposited on land or in water by an intermediate host (Roberts and Janovy, 2005). A species-specific larval stage then develops which, when ingested by the second intermediate host, is infectious to the definitive host

Primates may act as both definitive and intermediate hosts in the lifecycles of various species of cestode. For example, cystercerci (larvae) can encyst in the brain, liver or lungs of an intermediate host, causing serious pathology if left untreated (Roberts and Janovy, 2005). A study performed by Dunbar (1980) found that heavy infections with *Taenia* sp. in a population of *Theropithecus gelada* resulted in the death of many individuals. Species of cestode recovered from *Alouatta* sp. are restricted to the order Cyclophyllidea and include *Bertiella* sp. (Dunn, 1963), *Moniezia* sp. (Dunn, 1963), and *Raillietina* sp. (Thatcher and Porter, 1968; Dunn, 1963).

Trematoda

Organisms of the class Trematoda are commonly known as flukes. These parasites are often called "digenean trematodes", referring to the fact that generations alternate between sexually reproducing adults and asexually reproducing larvae (Roberts and Janovy, 2005). The lifecycle of all trematodes is complex and typically involves a mollusk as the first intermediate host and a vertebrate as the definitive host; secondary intermediate hosts (such as arthropods) are required in the lifecycles of some species. Adult trematodes produce an egg that hatches inside the mollusk intermediate host and reproduces asexually. A final larval stage (cercaria) is then released from the mollusk and encysts in vegetation or in a second intermediate host as a metacercaria, until ingested by a definitive host where development into adulthood occurs (Roberts and Janovy, 2005).

Digenean trematodes have the ability to cause severe pathogenesis within both the definitive and intermediate hosts. Within intermediate hosts such as ants and mollusks, larval stages may cause the host to act in a manner that increases the likelihood of

transmission to the definitive host (e.g. changing location) (Roberts and Janovy, 2005). Pathology in the definitive host usually presents in the bile ducts and liver. Livers of sheep infected with organisms from the family Dicrocoeliidae have been found to harbor up to 50,000 adult worms; severe infestations such as this may cause biliary dysfunction, bile duct inflammation, hepatocyte degeneration and liver necroses (Roberts and Janovy, 2005). *Controrchis biliophilus* infections have been reported from populations of *A. seniculus* (Gomes and Pinto, 1978), *A. palliata* (Stuart et al. 1998; Gonzalez et al. 1983) and *A. pigra* (Vitazkova and Wade, 2007); Stoner and Di Pierro (2006) report the presence of an unidentified fluke resembling *Controrchis* sp. in a population of *A. pigra* in Mexico.

Acanthocephala

Parasites of the phylum acanthocephala are commonly known as thorny-headed worms due to the structure of the proboscis. Acanthocephala infections are relatively uncommon in primates, possibly due to the fact that primates rarely ingest the appropriate intermediate hosts required by many species of acanthocephalan for lifecycle completion (Roberts and Janovy, 2005; Nunn and Altizer, 2006). All species of acanthocephala require at least two hosts for completion of the lifecycle and undergo a series of developmental stages before becoming infective to the definitive host. In the typical lifecycle, eggs expelled in the feces of a definitive host develop into an ancanthor and subsequently infect an arthropod intermediate host, in which development proceeds through the ancanthella stage. Upon ingestion by a second intermediate host the

ancanthella develops into a cystacanth, which is infective to the definitive host through ingestion of the intermediate host (Roberts and Janovy, 2005).

Similar to the larval stages of digenean trematodes, cystacanths of some species of acanthocephala have the ability to alter the behavior of the intermediate host, increasing the likelihood of ingestion by a definitive host (Moore, 1984). Infection with *Polymorphus paradoxus* in amphipod intermediate hosts causes changes in phototaxis, resulting in a decreased response to water disturbances and an increased susceptibility to predation by muskrat or duck definitive hosts (Maynard et al. 1996). Upon development into the adult stage, acanthocephalans have the ability to cause severe pathogenesis in definitive hosts.

Adult acanthocephalan species attach to the gastric cecum of the definitive host, and infections are often fatal. Penetration of the proboscis into the intestinal mucosa of the host, as well as the parasites' ability to reattach at preferred locations in the gut has resulted in complete perforation of the large intestine in some species of primates (Roberts and Jonovy, 2005). Although pathogenesis caused by many species of acanthocephalan can be severe, little research has been conducted examining the role that acanthocaphlans may play in reducing wildlife populations. There is one report of *Prosthenorchis elegans* infection in an individual *A. palliata* (Thatcher and Porter, 1968).

Susceptibility of hosts to parasitic infection

The density of infective parasite stages in the environment, contact rate between potential hosts and infective parasitic stages, and host resistance upon contact are all important in establishing the susceptibility of a host population to infection with a

particular parasite species (Scott, 1988). Conditions such as temperature, humidity and the number of adult parasites or infected hosts may influence the density of infective parasite stages in the environment. Large numbers of adult parasites and infected hosts will result in increased deposition of parasitic stages in the environment. The presence and distribution of endoparasitic infections among populations of non-human primates depends upon both inherent host traits and external environmental factors. The following section presents an overview of how endoparasitic infections may vary among alloprimates according to: age class and sex, membership in a particular group, group size, certain characteristics of home range, climatic factors, microhabitat and contact with human populations.

Age/Sex Class

The relationship between parasite species richness, prevalence and the age and sex of an individual may differ depending on both the parasite and primate species being considered (Nunn and Altizer, 2006). Studies of mountain gorillas (*Gorilla gorilla beringei*) in Rwanda have found no significant differences in parasite prevalence between males and females of different age groups (Sleeman et al. 2000). Moreover, similar protozoa and nematode prevalence values have been recovered from different age and sex classes of Angolan colobus monkeys (*Colobus angolensis palliatus*) in Kenya (Okanga et al. 2006). In populations of *Alouatta* sp., some studies report a higher prevalence of trematodes in adults than in juveniles or sub-adults (Stuart et al. 1998; Eckert et al. 2006; Vitazkova and Wade, 2006). In contrast, other studies of *Alouatta* sp. found no correlation between parasite prevalence and age (Stoner, 1996; Stuart et al. 1990). No

sex differences in macroparasite prevalence have been reported from populations of *Alouatta* sp. (Vitazkova and Wade, 2006; Stoner, 1993, 1996; Stuart et al. 1990, 1998).

Group Membership

Membership in a specific group has been correlated with susceptibility to parasitism in wild primate populations, with greater inter-group than intra-group variation in infection patterns. Vitazkova and Wade (2007) found that the most important factor in predicting whether an individual *A. pigra* would be infected with a gastrointestinal parasite was membership in a particular social group. Studies of other primate species corroborate this result; at Gombe Stream National Park in Tanzania, group membership was found to be the most significant factor influencing parasite prevalence in three groups of *Papio anubis* (Muller-Graf et al. 1997). A study of parasitism in provisioned versus non-provisioned troops of *P. anubis* recovered the heaviest loads of parasitic helminthes from a non-provisioned research group (Hahn et al. 2003). The non-provisioned group spent more time foraging and less time resting than the provisioned troop in this study, and a poorer quality diet is thought to account for the higher prevalence of helminth nematodes in non-provisioned animals. A study involving mantled howler monkeys (*A. palliata*) found differences in the intensity of parasitic infections between two monkey groups living in close proximity (Stoner, 1996). Variations in the intensity of infection were attributed to small differences in habitat and home range size and use.

Group Size

Social groups made up of many individuals are thought to facilitate the spread of directly transmitted parasite species leading to greater levels of parasite prevalence and diversity in large groups than in smaller social groups (Freeland, 1976; Anderson and May, 1979; Altizer et al. 2003b). As the number of individuals in a group increases and social networks expand, closer contact among group members is suspected to account for the increased intensity and prevalence of directly transmitted endoparasitic infections. Parasite species richness is usually greater in large social groups, as these aggregates provide a relatively larger environment for parasites to occupy (Anderson and May, 1979).

Research examining the association between group size and endoparasitic infection in primates has produced mixed results and may vary according to the transmission mode of each parasite. A correlation between group size and the spread and diversity of directly transmitted parasites has been documented for many social animal species including primates (Anderson and May, 1979; Altizer et al. 2003b; Cote and Poulin, 1995). A positive correlation between group size and the prevalence of intestinal protozoa has been documented in mangabeys (Freeland, 1979), and McGrew et al. (1989a) report a positive correlation between group size and nematode prevalence from a population of baboons at Gombe. Past research has found a positive association between group size among anthropoid primates (as measured by the number of females per group and/or social group size) and levels of helminth species richness. However, it should be noted that results from these studies were non-significant when phylogeny was included in analyses (Vitone et al. 2004; Nunn et al. 2003a).

Ranging

Previous studies focusing on parasitism in wild primate populations have found a correlation between levels of endoparasitic infection and home range size, day range length and intensity of range use. In a 2003 meta-analysis, Nunn and colleagues found that hosts whose ranges occupied a greater area were more likely to carry heavier parasite burdens than hosts that utilized smaller ranges (2003a). Relatively longer daily travel distances and the increased likelihood of encountering larger numbers of parasites and other animal species are thought to explain this association. Large home range sizes and day range lengths have been shown to partially account for increased nematode and complex-life cycle helminth diversity in anthropoid primate populations (Vitone et al. 2004). Whereas most nematodes are transmitted by close or non-close contact, or by biting arthropods, transmission of complex life-cycle helminthes typically involves trophic interactions and one or more intermediate hosts (Nunn and Altizer, 2006; Roberts and Janovy, 2005).

The restricted home range of *A. pigra* relative to other members of the genus *Alouatta* may be responsible for relatively low parasite diversity in this species (Vitazkova and Wade, 2006). At least seven helminth species have been recovered from a population of mantled howler monkeys (*A. palliata*) whose home ranges and group sizes are much larger than that of *A. pigra* (Stuart et al. 1998). In contrast, relatively small numbers of helminth species have been recovered from populations of *A. pigra* (Vitazkova and Wade, 2006 (n = 2); Eckert et al 2006 (n = 4); Stoner and Di Pierro 2006 (n = 4)).

As range size impacts macroparasitic diversity in hosts, the intensity of range use may lead to an increased risk of parasitism (Freeland, 1976, 1980). In a comparison of 119 primate species, helminth diversity was shown to correlate positively with range use intensity (Nunn and Tae-Won Dokey, 2006). An accumulation of infective stage parasitic stages in ranges that are used more intensively is thought to lead to an increased risk of infection in these areas. Intensive range use typically occurs in conjunction with territorial defense, and the fitness costs associated with defending a home range (vigilance, vocalization, inter-group aggression) may increase susceptibility to parasitosis in territorial species (Ezenwa 2004).

Habitat restriction resulting in home ranges containing many narrow corridors may also contribute to a high intensity of home range use. In two groups of *A. palliata,* the group that repeatedly used narrow arboreal pathways within their home range exhibited the highest intensity of nematode infections (Stoner, 1993). Members of the genus *Alouatta* often use the same arboreal pathways (Milton, 1980; Stuart et al. 1990; Stoner, 1993) and because many species of strongylid nematode infect hosts via the faecal-oral route, repeated pathway use may result in a higher likelihood of contact with contaminated foliage and more intense parasitic infections (Stoner, 1996).

Climatic Factors

Moist conditions tend to produce a favourable environment for the reproduction of many endoparasitic species and climatic factors such as proximity to water and rainfall patterns have been shown to impact parasitic infections in wild primate populations (Stoner, 1996; Stuart et al.1993, 1998; Stuart and Strier, 1995). The ingestion of fecally

contaminated food or water by hosts may account for the increased prevalence of water-borne parasites during the rainy season. In chimpanzees, the prevalence of the nematode *Oesophagostomum stephanostomum* increased in the rainy season, and re-infection occurred in synchrony with annual variation in rainfall (Huffman et al. 1997). Wet conditions facilitate the reproductive cycle of *O. stephanostomum*, and this is thought to account for the observed increase of re-infection during the rainy season.

Stoner (1996) examined parasitism in two groups of *A. palliata* occupying different habitat types, and recovered the highest prevalence of nematodes from the monkey group occupying alluvial tropical rainforest (compared to the group inhabiting dry deciduous forest). Other studies of *A. palliata* corroborate that moisture positively affects parasitism in this species of primate (Stuart et al. 1990). A high prevalence of nematode infections during the wet season, relative to the dry season, has been documented from a population of *A. pigra* in Belize (Eckert et al. 2006) and populations of woolly spider monkey (*Brachyteles arachnoids*) in Brazil (Stuart et al. 1993).

Microhabitat

A localized niche within the larger habitat occupied by a group of animals may possess characteristics different than that of the immediate surrounding area. These areas, known as microhabitats, may provide conditions favourable to the existence of large numbers of infectious stage endoparasitic organisms and increase the risk of infection to animals that come into contact with them (Freeland, 1976). Many species of endoparasites require specific conditions outside the definitive host in order to survive and reproduce. Temperature, oxygen content, moisture level and the amount of sunlight

present in the environment can all impact the proliferation of endoparasites (Appleton and Brain, 2008; Roberts and Janovy, 2005; Nunn and Altizer, 2006).

Soil and grass samples taken from a site in Amboseli, Kenya demonstrated that the area beneath sleeping groves of yellow baboons (*P. cynocephalus*) contained large numbers of infectious stage intestinal nematodes (Hausfater and Meade, 1982) from the feces of the baboons. The risk of endoparasitic infection in this microhabitat was relatively high compared to the rest of the home range and was avoided by the animals until the heightened risk of infection decreased to baseline levels.

Human Contact

The phylogenetic closeness shared by primates allows for the transmission of many zoonotic diseases between humans and non-human primate species. Almost 25% of micro and marcoparasitic infections found in wild primate populations have been identified from human populations (Pederson et al. 2005) and there are numerous accounts of cross-species disease transmission between humans and alloprimates (Stuart et al. 1990; McGrew et al. 1989a; Mudikikwa et al. 2001).

As habitat fragmentation and proximity to human settlements increases, the opportunity for endoparasitic transmission rises both within and between members of a species (Nunn and Altizer, 2006). Ecological changes resulting from human habitat disturbance (e.g. logging, agriculture) allows for increased transmission of parasitic infections, increased contact with novel host species, and may result in selection pressure leading to the dominance of new strains of pathogens adapted to new environmental conditions (Daszak et al. 2001). As expansion by humans into previously unexplored

areas leads to contact with novel zoonotic diseases, human environmental modification may drive the emergence of infectious diseases in human, domestic and wildlife populations (Daszak et al. 2001), as well as increase threats to both public health and non-human primate conservation (Muehlenbein, 2005).

Pathogens may be introduced to primate populations through many routes, and both humans and domestic animals are potential sources of infection (Tutin, 2000; Chapman et al. 2005a; Thompson et al. 2000). Anthropozoonotic disease transmission of helminthes and viruses have been reported from both chimpanzee and baboon populations in Gombe Stream National Park (Muller-Graf et al. 1997; McGrew et al. 1989a; Goodall, 1986). Anthropozoonotic transmission of *Giardia* sp. is speculated to occur in both populations of *G. g. beringei* in Uganda (Grazyk et al. 2002) and *A. pigra* in Belize (Vitazkova and Wade, 2007) through exposure to both humans and latrines. In a recent study, all groups of *A. pigra* infected with *Giardia sp.* inhabited an area occupied by labourers and researchers that contained latrines, suggesting zoonotic transmission of protozoa from humans or domestic animals to monkeys (Vitazkova and Wade, 2007). Human contact is also thought to be responsible for the transmission of *Ascaris lumbricoides* to an individual *A. pigra* living in close proximity to humans (Stuart et al. 1990).

The Effects of Endoparasitic Infection on Fitness and Behaviour

It is almost certain that parasites exert a strong selective pressure on the organisms that they infect, yet determining the actual fitness costs of parasitic infection in primates is difficult. Many parasite species cause general symptoms such as fever,

diarrhoea and reduced weight gain, which often cannot be directly attributed to infection with a specific parasite even if a positive diagnosis with infection is obtained (Scott, 1988). Although detection of visible diseases in wild animal populations is relatively straightforward, diseases that do not cause visible symptoms or result in mortality are notoriously hard to detect and quantify (Nunn and Altizer, 2006). In wild animal populations, reduced growth or delayed onset of sexual maturity due to parasitosis may only be detected through detailed, longitudinal studies performed on a set of known individuals (Scott, 1988).

Not all parasitic infections cause illness, but parasitosis can result in a variety of pathologies in primates (Kuntz, 1982). Manifestations of pathology in the host may result directly from the activities of the parasite (eg. intestinal obstruction from *Ascaris* sp.), or as a byproduct of the infection, as is the case with diarrhea resulting from malabsorption of water through the intestine (characteristic of *Giardia* sp.) (Olson and Buret, 2001). In isolation, non-pathogenic parasite species may have no impact on the fitness of a host, yet pathology may result from synergistic interactions caused by concurrent parasitic infections (Scott, 1988). Animals experiencing reduced nutritional status and or stress may be particularity vulnerable to infections of this type, as these individuals are at a disadvantage when attempting to mount an immune response (Isliker and Schurch, 1981; Vessey, 1964; Wiger, 1977).

Parasitosis may result in a number of physiological changes including fever, a reduction in iron levels, and reduced food intake and activity levels (Johnson, 2002; Huffman and Seifu, 1989; Krief et al. 2005). Fever can aid in recovery from infection by reducing parasite proliferation and increasing the immune response though increased

lymphocyte and antibody production and phagocytosis of infected cells (Johnson, 2002; Kluger, 1991)."Sickness behaviours" such as lethargy and a reduction in food intake are likely behavioural indicators of infection with a pathogen (Hart, 1990).

Parasites have the ability to exert a variety of selective pressures on the hosts that they infect, potentially altering individual host behaviors, the outcome of predation events and competitive interactions between conspecifics (Scott, 1988; Nunn and Altizer, 2006). Parasites that have an effect on the physical appearance and health of hosts may affect patterns of female mate choice and the ability of individuals to actively compete for sexual partners (Freeland, 1981b; Hamilton and Zuk, 1982). Host survival, patterns of movement and reproduction can all be impacted by infection with certain macroparasitic species (Scott, 1988).

In some cases, parasite infections are clustered in dominant animals, yet in other instances subordinate animals harbour the most parasite infections. During experimental tests, male mice infected with *Trichinella spiralis* consistently lost dominance trials to uninfected males (Rau, 1984; Freeland, 1981b). These data suggest that infection with certain parasite species may impair the ability of males to defend a territory, which may consequently compromise the survival of individual animals or groups. Although not all species of nematode are associated with subordinate animals, heavy infections in dominant animals may be the result of greater access to contaminated feeding areas, as is the case in some reindeer populations (Halvorsen, 1986).

While some parasite species may be associated with aspects of dominance, others influence patterns of movement. In laboratory experiments, mice infected with the nematode *Syphacia obvelata* exhibited decreased exploratory activity relative to

uninfected controls (McNair and Timmons, 1977), while spontaneous running activity has been detected in mice infected with *Toxocara canis* (Hay et al. 1985). Reduced or spontaneous host mobility has the potential to directly impair foraging and feeding behaviours as well as predator escape, all of which are essential to survival in wild animal populations.

Other parasite species can directly and indirectly affect host reproduction. Breeding pairs of American kestrels infected with the nematode *Trichinella pseudospiralis* exhibited delayed production of the first egg, lower total egg output and a lower egg hatch rate relative to uninfected pairs of birds (Saumier et al. 1986). The reproductive success of females may be directly impacted by parasitosis if infection influences the ability to rear young. During laboratory experiments involving female mice, infection with *T. spiralis* did not affect the number of young produced, but infected mothers did exhibit a considerable decrease in the survival of litters (Weatherly, 1971). Infection with the liver fluke *Fasciola hepatica* has been shown to indirectly affect reproduction in domestic sheep through the delayed onset of sexual maturity, and reduction in fertility (Hope-Cawdery, 1976; Hawkins and Morris, 1978).

Although parasitosis may be costly to individuals by increasing vulnerability to predation, decreasing opportunities for feeding, and impacting reproductive success in females, few accounts of "sickness behaviors" exist from wild primate populations. Documentation of sickness in wild primates is typically anecdotal, and there is a need for quantitative information, obtained from behavioral and parasite data, to provide additional information on the relationship between health and parasitism in primates (Nunn and Altizer, 2006).

Parasite Avoidance Strategies of Primates

With the exception of a few solitary or short-lived species, primates may be especially vulnerable to re-infection with infective fecal endoparasites. Behaviors that serve to reduce/avoid exposure to parasitic infections may constitute an alternative to physiological immunity, contributing to the increased fitness and survival of some individuals (Freeland, 1976, 1980; Gilbert, 1997). Human practices such as hand washing, sterilizing drinking water, and avoiding contact with sick individuals all serve to reduce exposure to infectious diseases through behavioral (rather than physiological) adaptations (Nunn and Altizer, 2006). Although the behavior of animals inflicted with parasitosis has long been viewed as a byproduct of the infection itself, behavioral strategies may serve as a first line of defense against endoparasitic infection prior to the initiation of an immune response (Hart, 1988; 1990, Keymer and Read, 1991; Nunn and Altizer, 2006).

Hypothesized parasite avoidance strategies used by primates include xenophobia (Moore, 2002), keeping newcomers on the periphery of the group (Freeland, 1976), behaviors involved in territorial defense (Loehle, 1995), selective defecation (including the differential use of defecation sites and defecation from low in the canopy), selective use of sleeping trees (Kowalewski and Zunino, 2005), and structured home range use in association with the amount of rainfall (Freeland, 1980). All of these behaviors may serve to decrease contact with infective stage parasitic larvae contained in the environment.

Xenophobia in primate groups may serve as a kind of "quarantine" whereby limited exchange between groups results in restricted exposure to new parasite species (Freeland, 1976; Moore, 2002). Keeping newcomers on the periphery of the group and

the maintenance of home ranges may all result in decreased exchange of parasites among groups. Although many forms of territorial defense that involve non-contact (vocalization, displaying) have been typically attributed to injury avoidance, these displays may also function to limit the exchange of pathogens among groups (Loehle, 1995). Similar to behaviors that serve to limit the inter-group exchange of parasites, certain behaviors involving defecation may serve to limit the intra-group exchange of parasites.

Selective Defecation

Many animals such as horses, sheep and various felids and canids use selective elimination as a way to avoid parasitism (Odberg and Francis-Smith, 1977; Crofton, 1958; Hart, 1990). Species inhabiting dens or nests on a continual basis may be at a higher risk of re-infection with endoparasites than species that differentially use sleeping sites within their ranges. The avoidance of microhabitats where the risk of infection with parasitosis is relatively high has also been documented in populations of Galápagos marine iguanas (Wikelski, 1999). Although many primate species are primarily arboreal and expelled feces drops from trees, contamination of foliage (potential food items) with infective-stage parasitic organisms contained in fecal matter may still occur. Behavioral strategies that serve to reduce contact with contaminated foliage and lessen exposure to parasitic larvae are suspected to occur in a number of primate species.

Kowalewski and Zunino (2005) examined the use of sleeping trees and selective elimination as a behavioral mechanism to avoid parasitic re-infection in a population of black howler monkeys (*A. pigra*). Results showed that over 20 days of study, monkeys

used the same trees for defecating and sleeping from six different sites. Also, monkeys were observed to rub their anuses on trees after defecating, a behavior observed in a population of *A. seniculus* as well (Braza et al, 1981). These findings suggest that selection of sleeping trees does not function to reduce exposure to endoparasitic infection in *A. pigra*. However, the behavior of defecating close to the ground, in areas of sparse understory, has been identified in a number of *Alouatta* sp. and may represent a behavioral strategy to reduce exposure to directly transmitted endoparasites (Gilbert, 1997; Stuart et al, 1990; Kowalewski and Zunino, 2005; Henry and Winkler, 2001).

A. seniculus (red howler monkeys) defecate selectively, in places where stool is more likely to fall to the ground without contaminating foliage (Gilbert, 1997). Individuals in this study moved away from the resting area, looked down prior to defecating, and were shown to avoid defecating on branches used for traveling and resting. Similar defecation behaviors have been documented in a population of *A. palliata* (mantled howler monkeys), in which individuals defecated from peripheral places in the canopy that were low to the ground (Henry and Winkler, 2001).

Yellow baboons (*P. cynocephalus*) in Amboseli demonstrate differential use of sleeping groves resulting in minimal contact with infective stages of nodular worms. Hausfater and Meade (1982) found the highest density of infective stage parasites in the soil beneath the sleeping groves of *P. cynocephalus* groups. By means of nematode larvae mortality schedules, it was established that the animals could decrease the probability of contact with infectious parasitic larvae by avoiding the reuse of sleeping groves during the 8.5 days required to reduce larval numbers to baseline levels (Hausfater and Meade, 1982). Observations of group movements recorded the mean return time

following any interval of continuous grove use as 9.1 days, supporting the hypothesis that temporal variation in sleeping grove use may decrease the risk of nodular worm infection.

Structured home range usage, in the form of avoidance of previously used areas during dry weather, has been proposed as a behavioral adaptation to endoparasitic avoidance used by *Cercocebus albigena* (mangabeys). Freeland (1980) examined *C. albigena* group movement patterns in relation to food availability and fecal contamination, and found that groups traveled further, used a larger area and exhibited less day-to-day overlap in area use during dry relative to rainy weather. An increase in feeding behavior was observed on days with rain and it is speculated that the rain helped cleanse the foliage of fecal matter, facilitating the intensive use of smaller feeding areas (Freeland, 1980). Animals in this study did not avoid fecal contamination of foliage during ranging. Later research on the relationship between weather and ranging in this species does not support the results of Freeland (1980), and suggests fruit availability as a major factor influencing the ranging patterns of *C. albigena* (Olupot et al. 1997).

Recent studies have failed to find a positive correlation between the risk of parasitism and sleeping site selection; factors such as predator avoidance and proximity to food resources may also influence the selection of sleeping sites (Hahn et al. 2003; Anderson and McGrew, 1984; Day and Elwood, 1999; Di Bitetti et al. 2000). A study of sleeping site selection in *Saguinus midas* (golden-handed tamarins) supported parasite avoidance (animals settled for a short time at numerous sleeping sites), but also showed predation and proximity to food to be factors impacting the selection of sleeping sites in this species (Day and Elwod, 1999). It has been suggested that pressure to select sleeping sites based on the risk of parasitism may only exist in terrestrial species, which are at a

greater likelihood of contacting parasites from the soil and vegetation beneath sleeping sites (Di Bitetti et al. 2000). Also, the role of sleeping sites is likely to be species-specific, and may even differ between populations of the same species (Nunn and Altizer, 2006).

Alleviation and Elimination of Endoparasitic Infection Through Diet

The majority of research involving parasite avoidance strategies in primates has focused on the ingestion of food items with zoopharmacological properties (Huffman et al. 1997). Parasite control strategies involving the ingestion of food items have been typically classified as "curative" (therapeutic) or "preventative" (prophylactic) (Phillips-Conroy, 1986; Lozano, 1991). The ingestion of foods for preventative purposes may be associated with the risk of parasitism, but not necessarily with the presence of parasites. In cases of therapeutic self-medication, only parasitized individuals are expected to consume curative foods that would not normally encompass part of the regular diet. The purpose of the consumption of curative foods may be to destroy parasites already established within the host or to alleviate the physical discomfort associated with parasitosis (Lozano, 1998; Huffman et al. 1997).

Several primate species have been described as exhibiting curative "self-medicative" behaviors, which serve to decrease endoparasitic loads or alleviate clinical symptoms associated with parasitic infections. Self-medicative behaviors of primates may include medicinal plant use and geophagy (Hart, 1990; Huffman and Wrangham, 1994; Huffman et al, 1997; Knezevich, 1998; Stoner and Di Pierro, 2006). A lack of detailed behavioral and health-related data from wild primate populations may have led

to the oversight of many therapeutic methods used to combat endoparasitic infection, as these behaviors are thought to be rare and may go unnoticed in studies not focusing specifically on individual health and dietary intake (Nunn and Altizer, 2006).

Various primate species may combat gastrointestinal parasitic infections through the ingestion of plants with medicinal properties (Huffman et al. 1997). Substantiation of this relationship includes the infrequent consumption of plants which are not a regular part of the diet, ingestion of plant material which provides no nutritional benefit, restriction of medicinal plant use to periods when the risk of parasitic infection is high, parasitic infection or illness at the time of medicinal plant use, and a positive change in this condition subsequent to ingestion of the plant in question. Leaf swallowing and bitter pith chewing have been identified in a number of great ape populations throughout Africa, and are both speculated to be behavioral adaptations used to combat endoparasitic infections (Huffman and Seifu, 1989; Wrangham and Nishida, 1983; Huffman et al, 1993; Huffman et al, 1997). A recent study by Stoner and Di Pierro (2006) found a correlation between the consumption of *Ficus* spp. and parasite intensity in *A. pigra*; individuals that spent a greater amount of time feeding on *Ficus* spp. had less intense parasite infections.

While selective foraging for specific plant foods has been chiefly documented in ape populations, the ingestion of clay soils, known as geophagy, has been recorded from numerous primate species (Krishnamani and Mahaney, 2000). Although little definitive evidence exists, geophagy is speculated to alleviate the symptoms and consequential debility caused by many endoparasitic infections. The practice of geophagy is widespread among human populations and is commonly used as an anti-diarrheal to treat

gastrointestinal aliments (Mahaney et al. 1995b, 1996b; Krishnamani and Mahaney, 2000; Knevevich, 1998). Many studies examining geophagy in primates have confirmed the presence of the clay minerals kaolinite and smectite in the soil consumed. Kaolinite is the primary ingredient in Kaopectate®, a pharmaceutical product for humans which functions as an anti-diarrheal and aids in the relief of gastrointestinal upset (Krishnamani and Mahaney, 2000). The absorptive properties of kaolinite and smectite suggest consumption of soils containing these products would produce the effect of hardening the stool (Knezevich, 1998; Krishnamani and Mahaney, 2000).

Geophagia has been documented from both *A. pigra* and *A. seniculus* (Bicca-Marques and Calegaro-Marques, 1994; Izawa, 1993). *A. seniculus* have been observed to ingest soils from salado or "salty" sites, as well as from arboreal termite nests (Izawa, 1993). Although the soils ingested by *A. seniculus* displayed relatively high pH values and may have functioned in the adjustment of gut pH, chemical analysis of soils from salados and arboreal termite mounds did not provide an explanation for soil consumption in this study (Izawa, 1993). A 1989 study documented a juvenile female black howler eating soil from an oven-bird nest (Bicca-Marques and Calegaro-Marques, 1994). Although soil from the nest was not analyzed, animals in this study were infected with large numbers of cestode worms and the subsequent geophagy may have decreased or provided relief from endoparasitic infection.

CHAPTER THREE: METHODS

Research Site and Study Population

The Monkey River research site is located in Belize, Central America and is north of the Monkey River at the Caribbean Sea (16° 21° N, 88° 29° W). The study area is approximately 86ha^2 of semi-evergreen broadleaf forest within 96km^2 of forested area (Fig.1). The average rainfall in Southern Belize is 457 cm per year and the average annual temperature is 26°C. During 2001 Monkey River was directly in the path of a category 4 hurricane that completely defoliated the forest (Pavelka et al. 2003; 2007). This event destroyed many keystone resources (e.g. *Ficus* sp.) and as a result, much of the current vegetation in the research site consists of pioneer and secondary-stage tree species such as *Cecropia peltata*, *Guazuma ulmifolia*, *Piper* spp. and *Miconia* spp.

The population of *A. pigra* in Monkey River has been studied since 1999 and occurs within a larger population of *A. pigra* in the Monkey River watershed. Prior to Hurricane Iris (2001), the site contained 53 monkeys in eight groups; as a result of the hurricane the monkey population experienced a dramatic reduction in numbers and only began to recover in 2005. During this research (January – June 2007), the site contained 31 monkeys in six groups; 17 individuals in four of these groups were chosen for study (Fig.1).

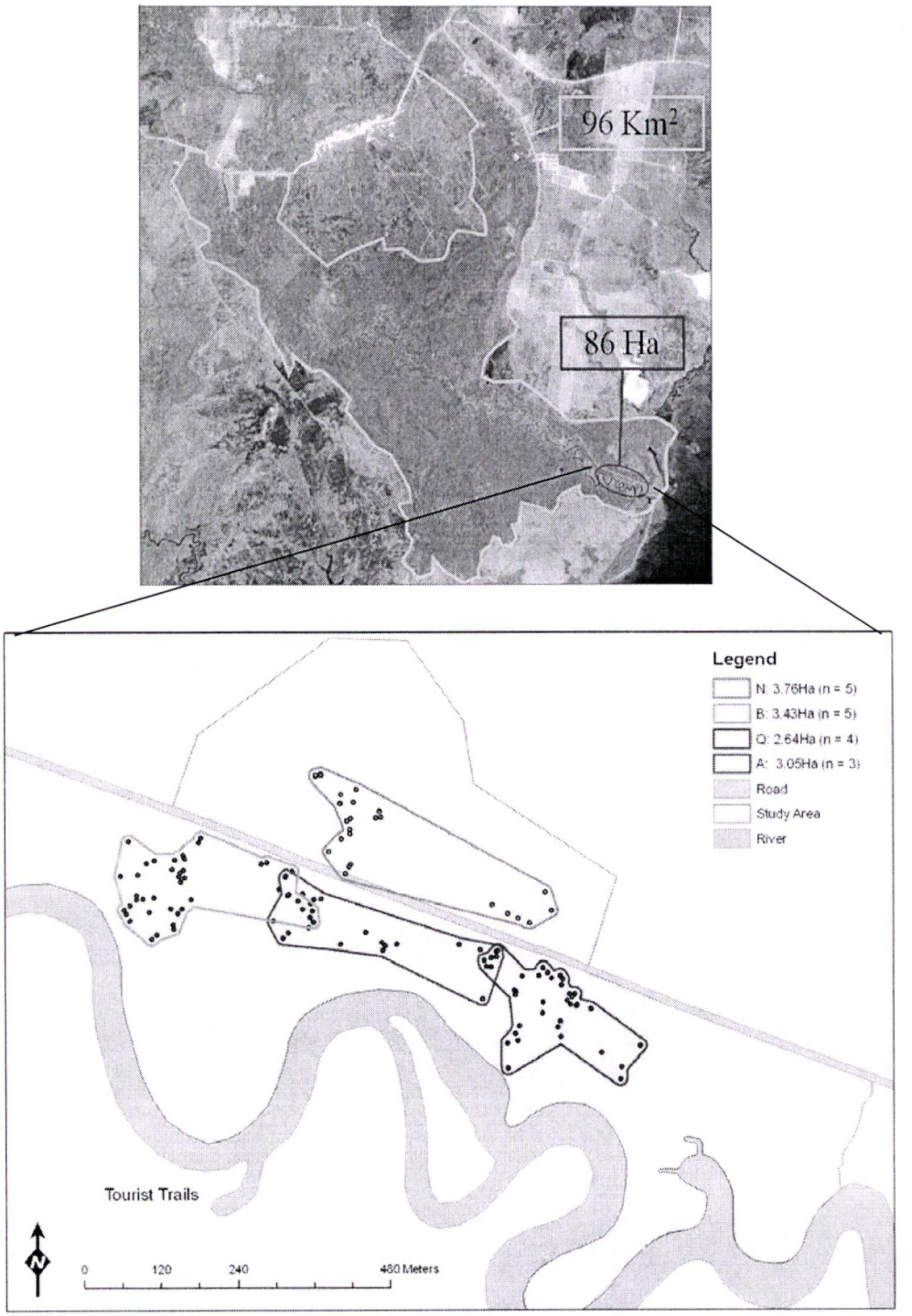


Figure 1: Map of Monkey River Watershed and Monkey River Study Site

Map depicting 86 ha study site located within 96 km² of forested area along the Monkey River in southern Belize, Central America. Inset: Map of study site showing the home range and number of individuals in each study group; tourist groups located south of river

The home range of each study group shown in Fig.1 was calculated using the multiple convex polygon method (MCP). Multiple convex polygon home range calculations were based on the outermost location points recorded for each group and may therefore over-estimate the actual area of each home range (Table 2).

Table 2: Home Range and Number of Individuals per Group

Group	# Individuals	MCP (Ha)
A	3	3.05
B	5	3.43
N	5	3.76
Q	4	2.64

Data Collection

Several types of data were collected to fulfill each of the research objectives for this study. Focal animal sampling supplied information on diet and activity and fecal samples were used to assess levels of parasitic infection. Records of group defecation were used to determine if a reduction in canopy height serves as a possible parasite avoidance strategy. To ascertain the impact of human contact on parasitism, the parasite prevalence of monkeys experiencing very little human contact (within the study site), was compared to that of another set of monkeys commonly visited by tourists. Rainfall data from Payne's Creek National Park was used to compare parasite prevalence between the wet and dry seasons.

Behavioral and fecal data were collected from 17 individuals living in four well-known study groups, ranging in size from three to five individuals (Table 2). Infants were not included in this study and as there were only two sub adults in the study groups, this age class was grouped with the adults for analysis. Groups were located by walking the road and trail systems and sometimes by following vocalizations. The sex of individuals was determined by inspection of the conspicuous genitalia characteristic of this species. Age categories (adult, subadult, juvenile, infant) were based on the physical size of individuals and sometimes by consulting birth records. Distinguishing characteristics such as facial scars or broken digits were used to identify individuals in the population.

Table 2: Study Group Compositions

Group	AM	AF	SM	SF	JM	JF	TOTAL
A	1	1	-	-	-	1	3
B	1	2	1	-	1	-	5
N	1	1	-	1	1	1	5
Q	1	1	-	-	-	2	4
TOTAL	4	5	1	1	2	4	17

Research followed a six-day schedule that sampled four study groups and several tourist-visited groups on the south side of the Monkey River. For four of the six days, half-day follows of the study groups were performed on a rotating basis that attempted to sample all groups and individuals equally. During half-day follows I collected fecal samples, behavioral data and fecal event data. One day of the research schedule was used as a "catch-up" day to collect data that we were unable to recover during the previous four days due to the inability to locate a specific study group, collect a sufficient number

of fecal samples or observe a group defecation event. Each data collection cycle, I spent one day traveling to the south side of the Monkey River to locate and collect fecal samples from monkeys frequently visited by tourists.

Tourist monkeys range ~3.5 km west of the mouth of Monkey River and are visited by tourists year-round. Lead by a local guide, groups of between 3-25 people visit these groups almost daily and it is common for guides to induce howling in the monkeys through vocal and mechanical (tree shaking) means. Although no physical contact between monkeys and humans has been reported, monkey groups commonly defecate upon these occasions (personal ob.), and tourists are occasionally soiled with monkey stool. The group compositions and locations recorded during the collection of fecal samples from tourist monkeys ensured that the fecal samples selected for analysis were from unique individuals.

Daily records were kept of the location and composition of all the groups encountered in the study site. I attempted to locate study groups either at dawn or early in the afternoon to try to ensure the observation of a group defecation event, which typically occurs after resting and before feeding. To obtain data on group defecation behaviors that may impact levels of parasitism, I recorded the behavior of the group and the distance from the ground upon the initial sighting. Any changes in canopy height or behavior after the initial sighting but prior to defecation were also noted. Upon seeing an individual defecate, the behavior, time and distance from the ground of the group were recorded. An attempt was also made to record the behavior and height of the group subsequent to defecation (See APPENDIX for data collection sheet).

To obtain data on the diet and activity of individuals, focal animal sampling was performed on adults, subadults and juveniles in the four study groups. During half-day follows, three 10-minute focal samples / hour were collected, and a 10% out of sight rule was employed. During social behavior the direction and age-sex class of interactants were recorded; when an animal was feeding I noted the location, plant species (when known) and plant part eaten.

Fecal samples were collected opportunistically from all adults, subadults and juveniles in the four study groups, in an attempt to obtain one fecal sample per individual for every data collection cycle. Fecal samples from tourist monkeys were recovered from a number of different groups and the samples chosen for analysis represent 24 unique individuals living in six groups. All fecal samples were collected immediately following defecation to ensure a positive match of the individual to the sample obtained. As individuals in the study population are easily identified, group sizes are relatively small, and individuals typically move down in the canopy to defecate, all fecal samples are known to correspond to the correct individuals. Feces were collected with wooden spatulas, and placed in plastic screw-top containers. Fecal samples typically fell to the forest floor and care was taken to avoid collection of feces contaminated by soil, flora or water. For each fecal sample collected, I recorded the individual, group, date, location, and individual height. At the end of each day, 2g of stool from each fecal sample was weighed using an electronic scale and placed in a 95% ethanol solution to be transported to the University of Calgary School of Veterinary Medicine for analysis.

Data Analysis

A total of 314 fecal samples were collected from individuals in four study groups (277 samples) and various tourist-visited groups (37 samples). For each study group I analyzed one fecal sample per individual per month (Q group =24 samples; A group =17 samples; B group =28 samples; N group =24 samples; tourist monkeys =24 samples), resulting in the overall analysis of 117 samples. All samples were examined for the presence of *Giardia* sp. cysts and helminth eggs and larvae.

Three different measures of parasitism were calculated for the purpose of this study. **Parasite abundance** was calculated as the number of eggs / gram of feces analysed from both infected and uninfected fecal samples (Margolis et al. 1982). To calculate this measure, the average abundance (with each macroparasite species) was calculated for each individual by counting the number of eggs recovered from both positive and negative fecal samples. The mean abundance of each parasite species was calculated for groups and for the population by averaging the mean abundance of all group members or study animals. Counting parasite eggs in fecal samples is commonly used as to assess of the severity of parasite infection, yet the number of eggs shed in the feces may under-represent level of parasites in the gut. Despite the constraints of this method, determining levels of parasite infection based on the quantity of eggs shed in the feces is one of the best tools available to assess gastrointestinal parasite infections in wild primates.

Parasite prevalence is typically defined as the percent of hosts infected with a parasite species and is calculated by dividing the number of positive hosts by the number of hosts sampled (Margolis et al. 1982). As multiple fecal samples were collected from

known individuals in this study, the parasite prevalence was assessed using two different methods. **Host prevalence** was determined by dividing the number of hosts testing positive for a given parasite (at any time during the study) by the total number of hosts sampled (# hosts infected / # hosts sampled). **Sample prevalence** was calculated by dividing the number of fecal samples testing positive for a given parasite species by the total number of fecal samples analyzed (# fecal samples positive / # fecal samples analyzed). Whereas host prevalence was calculated in the classic fashion (# hosts infected / # hosts sampled) and is a group or population measure, as multiple fecal samples were collected from each individual, sample prevalence is a measure that can apply to individuals or be averaged for the group or population.

To obtain helminth egg counts, fecal samples were analyzed using a modified Wisconsin quantitative sugar centrifugation technique (according to the procedure provided by the University of Saskatchewan, Dept. of Veterinary Medicine) in the laboratory of Dr. Susan Kutz at the University of Calgary School of Veterinary Medicine. 1 gram of feces was measured from each 2g sample and filtered through two layers of cheesecloth into a plastic cup. Feces were then centrifuged in a 16 x 100mm tube for 4-minutes at 1500 rpm. After centrifuging, the supernatant was decanted and the sediment was vortexed in 5ml of Sheather's solution. Sheather's solution was then added to the tube until it formed a convex meniscus. A 18mm x 18mm coverslip was then placed on the meniscus and the tube was centrifuged for another 4-minutes at 1500 rpm. After centrifuging, the coverslip was pulled straight up, transferred to a labelled slide, and scanned under 100x magnification using a compound microscope. All helminth eggs and larvae were identified based on their size and morphology. Photographs and

measurements were taken using an ocular micrometer fitted to a compound microscope using Infinity Analyze imaging software.

Aqua-Glo G/C direct comprehensive kits (available from Waterborne Inc.) were used to test fecal samples for the presence of *Giardia* sp. cysts and oocysts. These kits bind a fluorescein labelled antibody to the cysts/oocysts so that they can be observed under a fluorescence microscope. I prepared all the slides to be analyzed at the Calgary Laboratory of Microbiology (CLM) the same day as preparation. A small amount of fecal sediment was smeared in each slide well and allowed to dry completely. One drop of Aqua-Glo reagent was added to each well and slides were incubated in a humid chamber at room temperature for 40 minutes; slides were then rinsed with a wash buffer. One drop of counter-stain was added to each well and slides were incubated at room temperature for one-minute. After rinsing with wash buffer, each slide was allowed to air dry at a slant. One drop of mounting medium was added to each well and 20mm x 20mm coverslips were applied and sealed with nail polish around the edges. All slides were observed under 400x magnification using a fluorescence microscope at CLM. Samples were compared to positive controls to identify the "apple-green" glow and specific shape of *Giardia* sp. cysts/oocysts. Due to their small size, only the presence/absence of *Giardia* sp. cysts was determined for each sample.

All DNA extraction, PCR amplification and sequencing analysis for *Giardia* sp. was performed at Murdoch University (Australia) in the laboratory of Dr. Andrew Thompson, with assistance from Unaiza Parker. With the 1g of feces remaining from each fecal sample, DNA was extracted using QIAamp DNA Stool Mini Kit (Qiagen, Germany) according to the manufacturer's protocol, employing the adjustments

mentioned in Parkar et al. (2007). A fragment of the SSU rDNA for *Giardia* was amplified by a nested PCR using previously described primers. The primary reaction utilized the forward primer, RH11 (5` CAT CCG GTC GAT CCT GCC 3`) and reverse primer, RH4 (5`AGT CGA ACC CTG ATT CTC CGC CAG G 3`) described by Hopkins et al. (1997). The primers, GiarF (5`GAC GCT CTC CCC AAG GAC 3`) and GiarR (5` CTG CGT CAC GCT GCT CG 3`) described by Read et al. (2002) were used in the secondary reaction. Both reactions were performed under conditions previously described by Santín et al. (2007). PCR products were also purified from reactions using the Wizard SV Gel and PCR Clean-Up System (Promega Corporation) according to the manufacturer's kit protocol. The PCR products were sequenced in the direction using an ABI 3730 capillary sequencer. Sequences were analyzed using FinchTV and compared with previously published sequences from GenBank using the BLAST 2.2.9 program (<http://www.ncbi.nlm.nih.gov/blast).

Statistical Analysis

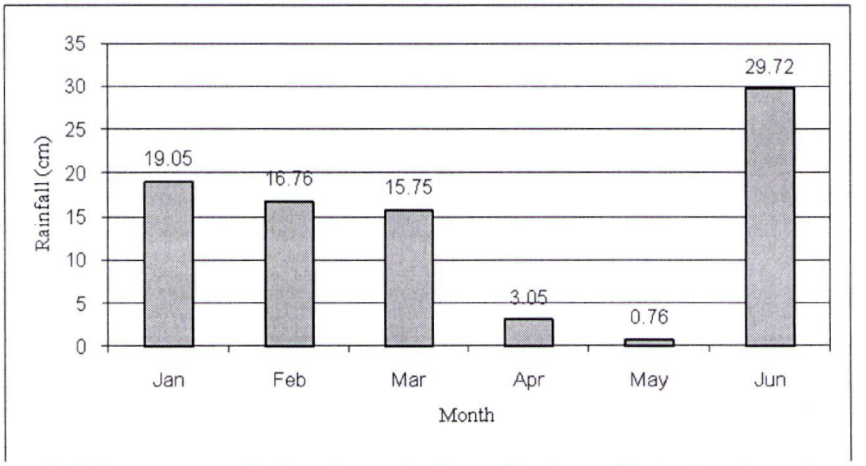
When performing statistical analysis on this dataset, testing was done to ensure all assumptions were met and the appropriate statistical tests were selected for use. A confidence interval of 95% and an alpha value of 0.05 were assumed for all statistical tests performed. To compare the prevalence of parasites between age classes, sexes or among groups, prevalence was calculated as the percent of fecal samples from an individual that tested positive for a given parasite species (sample prevalence).

Differences in parasite prevalence between age classes and sexes were compared using non-parametric Mann-Whitney U tests and variation in parasite prevalence among

groups was examined using non-parametric Kruskal-Wallace tests. The exception to this case occurred when comparing parasite prevalence between tourist and study monkeys. Multiple fecal samples were collected from each study monkey and each tourist monkey was represented by only one fecal sample. To correct for this problem, a random fecal sample was chosen from each study individual by rolling a dice and a Chi-square test was used for analysis.

Wilcoxon Signed Rank tests were used to determine the relationship between seasonality and parasite prevalence. All rainfall data used in this study was obtained from records kept by Payne's Creek National Park. Fecal samples from each individual were classified as 'wet' or 'dry' season samples based on the month they were collected; January – March were classified as wet months, and April – June as dry months, partially based on the amount of precipitation that fell during each month (Fig. 2). Based on the rainfall data presented in Figure 2 June is a very wet month and this month is typically the beginning of the wet season. Although the total rainfall recorded in June was the highest for the entire study period (29.72 cm), the vast majority of this rain fell after research had concluded (June 15th). As information on daily rainfall was not available, and as all data collection in June occurred under very dry conditions, June was considered as a dry month for the purpose of seasonal analysis.

Figure 2: Monthly Rainfall at Monkey River, January – June 2007



Monthly rainfall (cm) recorded at Payne's Creek National Park, Southern Belize. Rainfall in June occurred after the completion of this study

Linear regressions were used to assess if parasite abundance (macroparasite species), or the percent of samples testing positive for *Giardia* sp. had an impact on the amount of time individuals spent feeding and resting. Simple regressions were also used to determine the effect of *Cecropia peltata* ingestion on *Controrchis* sp. abundance.

CHAPTER FOUR: RESULTS

This study recovered four species of gastrointestinal parasites from the population of *A. pigra* in Monkey River. Variation in the prevalence of endoparasitic infections was documented for age classes, sex classes and groups. The influence of parasitic infection on the amount of time individuals spent resting and feeding was examined to determine the potential effects of parasitosis on behaviour. The prevalence of parasitic infections was compared by season and the relationship between parasite prevalence and contact with humans was examined. To ascertain the possible route of infection of *Controrchis* sp. to the monkeys, the percent of time spent feeding on *Cecropia peltata* was compared to *Controrchis* sp. abundance among individuals. In conjunction with information on parasitosis in each group, records of group defecation were used to assess possible parasite avoidance strategies present in the study population.

Parasite Species Recovered

Four macroparasites were recovered (*Controrchis* sp. [Dicrocoeliidae], *Trichostrongylid* sp. [Strongyloididae], *Trichuris* sp. [Trichuridae]), along with one microparasite (*Giardia duodenalis* Assemblage B [Hexamitidae]) (Table 3). *Controrchis* sp. was present in 43 / 93 fecal samples (sample prevalence) and 13 / 17 individuals (host prevalence). A total of 154 *Controrchis* sp. cysts were recovered, resulting in an abundance of 1.656 eggs / gram (in infected and uninfected feces). The average size of *Controrchis* sp. eggs was 38.605 x 23.803 μm (N=8) (Fig.3). *Trichostrongylid* sp. was recovered from 19/93 fecal samples and 9 / 17 individuals. A total of 30 *Trichostrongylid* sp. eggs were recovered, resulting in an abundance of 0.323 eggs/gram. The average

Trichostrongylid sp. egg size was 84.123 x 46.269 μm (N=17) (Fig.3). *Trichuris* sp. eggs were recovered from 3 / 93 fecal samples and 3 / 17 individuals. A total of six *Trichuris* sp. eggs were recovered, resulting in an abundance of 0.065 eggs/gram. Because so few cysts were found from a limited number of individuals, no eggs were measured. *G. duodenalis* cysts were recovered from 42 / 93 fecal samples and 12 / 17 individuals.

Table 3: Overall Prevalence and Abundance of Parasites Recovered

Parasite species	Measure	TOTAL (All groups)
Controrchis sp.	Sample Prevalence	46.23% (43/93)
	Host Prevalence	76.47% (13/17)
	Average Sample Prevalence	47.65%
	Mean Abundance	1.656 eggs/ gram
Trichostrongylid sp.	Sample Prevalence	20.43% (19/93)
	Host Prevalence	52.94% (9/17)
	Average Sample Prevalence	19.47%
	Mean Abundance	0.323 eggs/gram
Trichuris sp.	Sample Prevalence	3.23% (3/93)
	Host Prevalence	17.65% (3/17)
	Average Sample Prevalence	4.06%
	Mean Abundance	0.065 eggs/gram
Giardia duodenalis	Sample Prevalence	45.16% (42/93)
	Host Prevalence	70.59% (12/17)
	Average Sample Prevalence	45.29%

Sample Prevalence (fecal samples testing positive / fecal samples analyzed) (n=93), host prevalence (# hosts infected / # hosts sampled) (n=17), average prevalence (average of fecal samples positive) and mean abundance (eggs / gram of infected and uninfected fecal samples) of gastrointestinal parasites recovered.

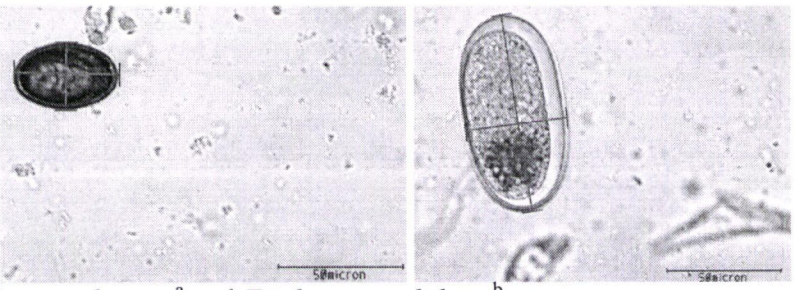


Figure 3: *Controrchis* sp. [a] and *Trichostrongylid* sp. [b] eggs

[a] (41.67 x 26.35μm) [b] (85.68 x 44.87μm)

The mean abundance of the different parasite species recovered suggests low levels of parasitic infection in the study population. The only parasite found to occur at a quantity of >1 eggs / gram of feces was *Controrchis* sp. (1.656 eggs / gram). Although necropsies are generally required to ascertain representative levels of parasitic infection in wild animals, and the shedding schedules of fecal parasites may vary, results from this study suggest that individuals in the study population suffered from relatively mild gastrointestinal parasitic infections.

Age and Sex Variation in Parasitism

The individual sample prevalence of *Controrchis* sp. differed significantly between adults and juveniles; a median of 80.00% (± 33.25) of fecal samples recovered from adults tested positive for *Controrchis* sp. and a median of 16.66% (± 25.09) of fecal samples from juveniles were positive for *Controrchis* sp. (Fig.4). *Controrchis* sp. prevalence did not vary between sexes and the individual sample prevalence of *G. duodenalis*, *Trichostrongylid* sp. and *Trichuris* sp. did not differ significantly between males and females, or between adults and juveniles (Table 4).

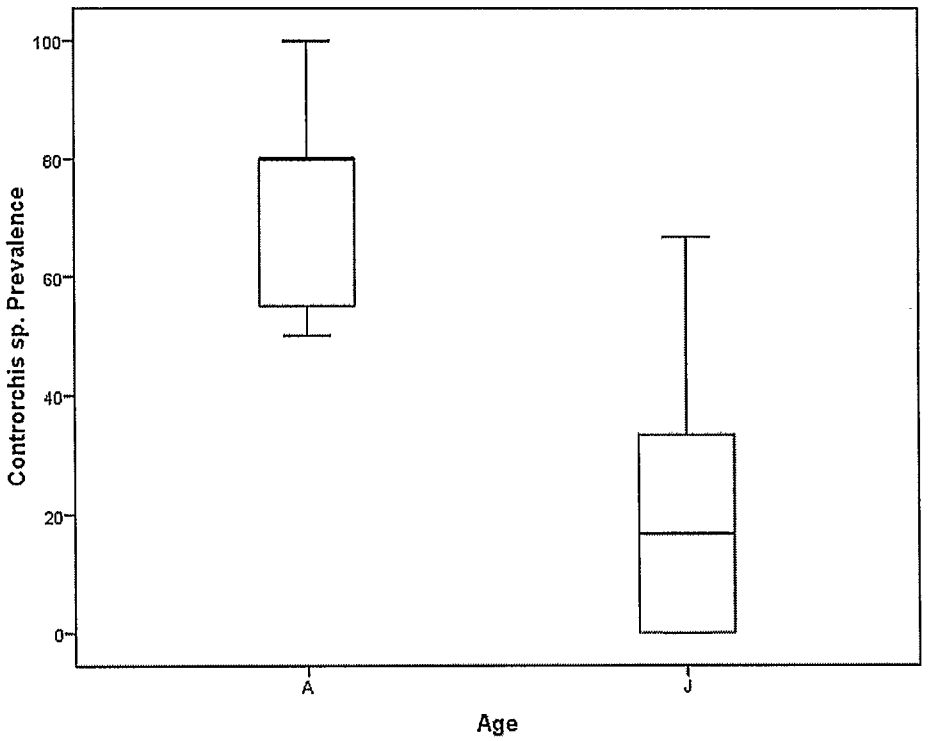


Figure 4: Median *Controrchis* sp. Prevalence by Age Class

The median sample prevalence (fecal samples testing positive / fecal samples analyzed) and standard deviation of *Controrchis* sp. for adults (A = 11) and juveniles (J = 6).

Table 4: Age and Sex Class Variation in Parasite Prevalence

Parasite Prevalence	Demographic	
	Age	Sex
Trichostrongylid sp.	$U = 29.00, P = 0.671$	$U = 29.50, P = 0.570$
Trichuris sp.	$U = 32.50, P = 0.940$	$U = 34.00, P = 0.883$
Controrchis sp.	$U = 12.50, P = 0.037$*	$U = 33.00, P = 0.843$
Giardia duodenalis	$U = 25.50, P = 0.442$	$U = 26.00, P = 0.370$

The sample prevalence (fecal samples testing positive / fecal samples analyzed) of each parasite species was assessed by age (adults vs. juveniles) and by sex (males vs. females) using Mann-Whitney-U tests (n =17).

Group Variation in Parasitism

Table five summarizes the variation in parasite prevalence and abundance of endoparasites among the four research groups. The sample prevalence of *Controrchis* sp. and *G. duodenalis* were similar among three of the four research groups (A, B, N), yet neither of these parasites were recovered from Q group; *Trichostrongylid* sp. was the only parasite detected from this group. *Trichuris* sp. eggs were found in fecal samples from groups B and N, but occurred at too low an abundance for significant differences in infection levels to exist among groups (0.065 eggs / gram). With the exception of *Controrchis* sp. (0.941- 4.375 eggs / gram) parasite intensities were all less than 1 egg / gram of feces.

Table 5: Prevalence and Abundance of Parasites Recovered

Parasite	Measure	Group				TOTAL
		Q	A	B	N	(All groups)
Controrchis sp.	Sample Prevalence	0.00% (0/24)	47.05% (8/17)	60.71% (17/28)	75.00% (18/24)	46.23% (43/93)
	Host Prevalence	0.00% (0/4)	100% (3/3)	100% (5/5)	100% (5/5)	76.47% (13/17)
	Average Prevalence*	0.00%	48.88%	61.33%	71.80%	47.65%
	Mean Abundance	0.00	0.941	1.178	4.375	1.656 eggs/ g
Trichostrongylid sp.	Sample Prevalence	20.83% (5/24)	29.41% (5/17)	28.57% (8/28)	4.16% (1/24)	20.43% (19/93)
	Host Prevalence	50% (2/4)	67% (2/3)	80% (4/5)	20% (1/5)	52.94% (9/17)
	Average Prevalence	20.83%	27.77%	30.00%	3.33%	19.47%
	Mean Abundance	0.375	0.471	0.393	0.083	0.323 eggs/g
Trichuris sp.	Sample Prevalence	0.00% (0/24)	0.00% (0/17)	3.57% (1/28)	8.33% (2/24)	3.23% (3/93)
	Host Prevalence	0.00% (0/4)	0.00% (0/3)	20.00% (1/5)	40.00% (2/5)	17.65% (3/17)
	Average Prevalence	0.00%	0.00%	3.33%	10.66%	4.06%
	Mean Abundance	0.00	0.00	0.036	0.208	0.065 eggs/g
Giardia duodenalis	Sample Prevalence	0.00% (0/24)	58.82% (10/17)	67.86% (19/28)	54.16% (13/24)	45.16% (42/93)
	Host Prevalence	0.00% (0/4)	100% (3/3)	100% (5/5)	80.00% (4/5)	70.59% (12/17)
	Average Prevalence*	0.00%	60.00%	68.67%	49.33%	45.29%

Sample prevalence (fecal samples testing positive / fecal samples analyzed); host prevalence (# hosts infected / # hosts sampled); average prevalence (average of fecal samples positive per group); mean abundance (eggs / gram of positive and negative fecal samples [n = 93]) of gastrointestinal parasites recovered (17 individuals: Q=4; A=3; B=5; N=5).

The difference between calculating parasite prevalence based on the number of fecal samples testing positive for a given parasite species and based on the number of hosts testing positive is demonstrated in Table 5. For example, when prevalence is calculated based on the number of hosts that tested positive for a parasite at any time over the period of the study, *Controrchis* sp. prevalence appears to be 100% in three of the four research groups. This measure does not take into account that *Controrchis* sp. eggs were recovered from only 47-75% of fecal samples obtained from individuals in these groups.

The median sample prevalence of *G. duodenalis* differed significantly among groups (Fig. 5). No *G. duodenalis* was recovered from Q group, which a post-hoc test (Mann Whitney-U) confirmed was significantly lower than the median *G. duodenalis* prevalence of the other groups (60.00% ± 31.97 [N group], 50.00% ± 17.32 [A group], 60.00% ± 22.19 [B group]). The average prevalence of *Controrchis* sp. also differed significantly among the study groups (Fig.6). Q group had no *Controrchis* sp. infections, which a post-hoc test (Mann Whitney-U) determined was significantly lower than the median *Controrchis* sp. prevalence of groups B and N, from which a median of 66.66% ± 26.73 and 80.00% ± 24.68 of fecal samples tested positive for *Controrchis* sp., respectively. The prevalence of *Trichostrongylid* sp. did not differ significantly among the study groups, nor were there any differences among groups in *Trichuris* sp. prevalence (Table 6).

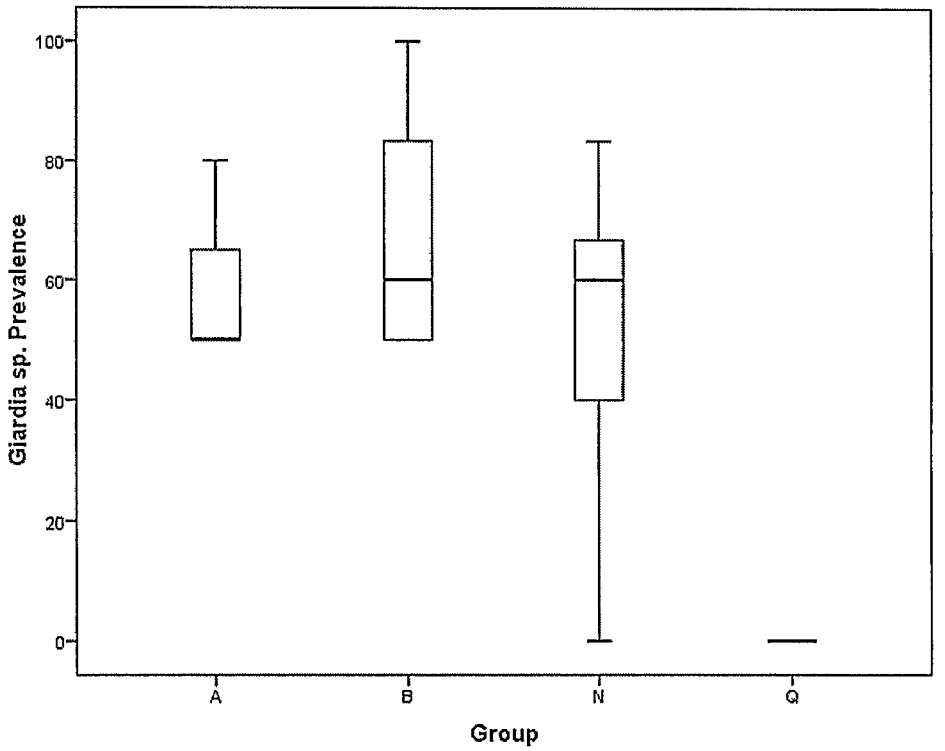


Figure 5: Median *Giardia duodenalis* Prevalence by Group

The median sample prevalence (fecal samples testing positive / fecal samples analyzed) and standard deviation of *Giardia* sp. by group (Q = 4 individuals, 24 fecal samples; N = 5 individuals, 24 fecal samples; A = 3 individuals, 17 fecal samples; B = 5 individuals, 28 fecal samples)

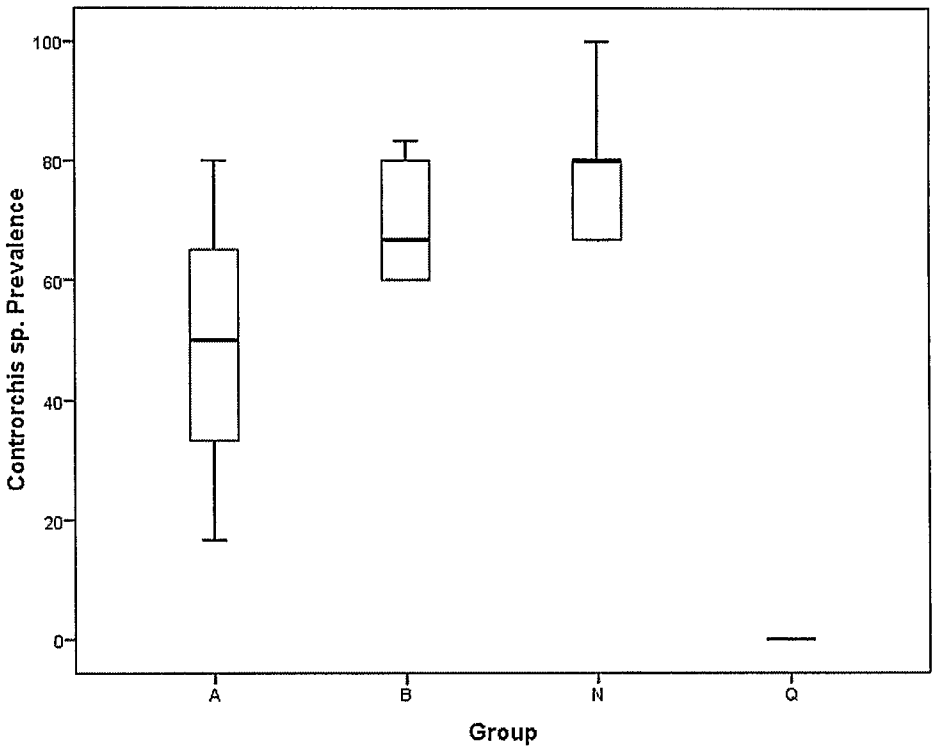


Figure 6: Median *Controrchis* sp. Prevalence by Group

The median sample prevalence (fecal samples testing positive / fecal samples analyzed) and standard deviation of *Controrchis* sp. by group (Q = 4 individuals, 24 fecal samples; N = 5 individuals, 24 fecal samples; A = 3 individuals, 17 fecal samples; B = 5 individuals, 28 fecal samples)

Table 6: Average Parasite Prevalence by Group

Prevalence	Group
Trichostrongylid sp.	Kruskal-Wallace (χ^2=4.501, df=3, p=0.212)
Trichuris sp.	Kruskal-Wallace (χ^2=3.351, df=3, p=0.341)
Controrchis sp.	Kruskal-Wallace (χ^2=9.733, df=3, p=0.021)*
Giardia duodenalis	Kruskal-Wallace (χ^2=8.449, df=3, p=0.038)*

Average of fecal samples testing positive for each parasite species by group (Q = 4 individuals, 24 fecal samples; N = 5 individuals, 24 fecal samples; A = 3 individuals, 17 fecal samples; B = 5 individuals, 28 fecal samples)

Parasitism and Behaviour

Parasitosis may cause a number of pathologies in infected hosts, including malaise, diarrhea, and a change in appetite (Johnson, 2002; Huffman and Seifu, 1989; Krief et al. 2005). This study investigated whether the abundance of any parasite species recovered was related to the amount of time an individual spent resting or feeding. 88 hours of focal animal data were collected during this study from 17 individuals distributed among the four study groups. There was no significant relationship between the abundance of any macroparasite species, or the average *G. duodenalis* prevalence and the amount of time spent resting or feeding by individuals; the exceptionally low overall parasite abundances recovered from the study population may partially account for this result (Table 7).

Table 7: Parasitism and Behaviour

Parasite	Behaviour	
	Inactivity	Feeding
Trichostrongylid sp.	F-ratio=0.009, df=16, p=0.926, r^2=0.001	F-ratio=3.491, df=16, p= 0.081, r^2=0.189
Trichuris sp.	F-ratio=1.151, df=16, p=0.300, r^2=0.071	F-ratio=1.657, df=16, p=0.217, r^2=0.099
Controrchis sp.	F-ratio=1.739, df=16, p= 0.207, r^2=0.104	F-ratio= 3.288, df=16, p=0.090, r^2=0.180
Giardia duodenalis	F-ratio=0.201, df=16, p=0.660	F-ratio=1.838, df=16, p= 0.195, r^2=0.109

Macroparasite abundance (number of eggs / gram of positive and negative fecal samples [n = 93]) and average prevalence (average of fecal samples testing positive) of *Giardia* sp. versus time spent resting and feeding by individual (n = 17)

Parasitism and Seasonality

The sample parasite prevalence did not vary by season for any of the species recovered (Table 8). The fact that many individuals were not infected with *Trichuris* sp. led to a high frequency of zeros and many ties in the Wilcoxon Signed-Rank test. This lowered the *n* and made comparisons of *Trichuris* sp. between seasons impossible. As was expected the host prevalence of all parasites was higher in the wet season, yet as there was only one measure of host prevalence for each season, differences in host prevalence between the seasons could only be analyzed descriptively (Fig.7).

Table 8: Sample and Host Prevalence by Season

Parasite	Prevalence	Season		Wilcoxon Signed-Rank Test
		Wet	Dry	
Giardia duodenalis	Sample	25/49 (51.02%)	17/44 (38.63%)	$n = 10$, $Z = -0.975$, $P = 0.329$
	Host	12/17 (70.58%)	9/17 (52.94%)	
Controrchis sp.	Sample	24/49 (48.97%)	19/44 (43.18%)	$n = 11$, $Z = -2.240$, $P = 0.823$
	Host	13/17 (76.47%)	9/17 (52.94%)	
Trichostrongylid sp.	Sample	15/49 (30.61%)	4/44 (9.09%)	$n = 7$, $Z = -1.364$, $P = 0.172$
	Host	9/17 (52.94%)	3/17 (17.64%)	
Trichuris sp.	Sample	3/49 (6.12%)	1/44 (2.27%)	N / A
	Host	3/17 (17.64%)	1/17 (5.88%)	

Sample prevalence (fecal samples testing positive / fecal samples analyzed) and host prevalence (# hosts infected / # hosts sampled) of parasites recovered in the wet (Jan – Mar) and dry (Apr-Jun) seasons. Wet season fecal samples ($n = 49$) and dry season fecal samples ($n = 44$) from 17 hosts

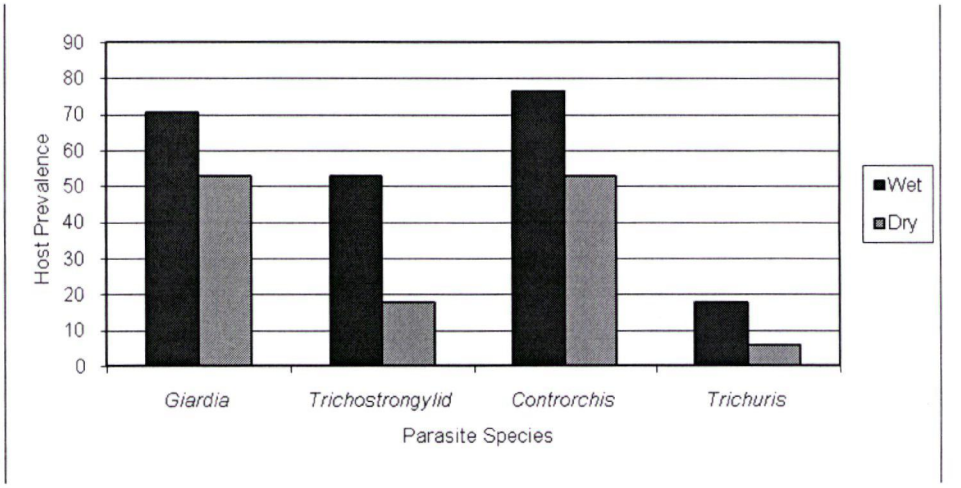


Figure 7: Host prevalence in wet and dry seasons

Host prevalence (# hosts infected / # hosts sampled [n = 17]) of parasites recovered in the wet (Jan – Mar) and dry (Apr-Jun) seasons. Wet season fecal samples (n = 49) and dry season fecal samples (n = 44) from 17 hosts

Parasitism and Human Contact

The parasite prevalence of monkeys commonly visited by tourists (n = 24) was compared to that of individuals within the study site (n = 17) to examine the relationship between contact with humans and endoparasitic infection. Due to the fact that each study individual was represented by ~ six fecal samples, while each tourist monkey was represented by one fecal sample, the likelihood of finding a study individual infected with a parasite was considerably higher. To correct for this problem one fecal sample was chosen for analysis from each study monkey by rolling a dice; the numbers on the dice corresponded to a fecal sample from one of the six months of study. Although variation in the prevalence of *G. duodenalis* and *Trichuris* sp. between tourist and study monkeys approached significance, there were no significant differences in the prevalence of any parasite species between tourist and study monkeys (Table 9).

Table 9: Host Prevalence Between Study and Tourist Monkeys

Parasite	Human Contact		Pearson Chi-Square
	Research (low)	Tourist-Visited (high)	
Giardia duodenalis	5/17 (29.41%)	14/24 (58.33%)	$\chi^2 = 3.347$, df= 1, p=0.067
Controrchis sp.	9/17 (52.94%)	7/24 (29.16%)	$\chi^2 = 2.364$, df =1, p=0.124
Trichostrongylid sp.	5/17 (28.41%)	4/24 (16.67%)	$\chi^2 = 0.943$, df= 1, p=0.331
Trichuris sp.	2/17 (11.76%)	0/24 (00.00%)	$\chi^2 = 2.968$, df=1, p=0.085

Host prevalence (# hosts infected / # hosts sampled) of parasites recovered from study monkeys ($n = 17$) and tourist monkeys ($n = 24$)

The Transmission of *Controrchis* sp. Through Diet

Controrchis sp. was the only parasite recovered that has an indirect lifecycle, requiring an arthropod second intermediate host for transmission to the definitive host (Krull and Mapes, 1953). Of the four parasites recovered, *Controrchis* sp. was found to exist at the highest abundance in the study population (1.656 eggs/gram) and the abundance of *Controrchis* sp. among individuals ranged from 0.00 eggs/ gram to 7.80 eggs / gram.

As arthropods are not typically contained in the diet of *A. pigra*, I investigated the possible source of *Controrchis* sp. infection as ants contained on *Cecropia peltata*, a plant species commonly fed upon by the monkeys. *C. peltata* has a symbiotic relationship with an arthropod (*Azteca* sp.), a possible intermediate host for *Controrchis* sp. (Vitazkova, 2005). As *A. pigra* rarely come to the ground, the accidental ingestion of *Azteca* sp. when feeding on *C. peltata* may account for the presence of *Controrchis* sp. in the population. Overall, 11.60% (2.33 hrs) of time was spent feeding on *C. peltata*, with

individuals spending 0.00% - 27.15% of time ingesting items from this tree. As predicted, a higher percentage of *C. peltata* in the diet of individuals was shown to result in more abundant *Controrchis* sp. infections (F-ratio=5.918, df=16, p=0.028, r^2=0.28) (Fig. 8).

The amount of time spent feeding on *C. peltata* also differed significantly among study groups (χ^2=7.858, df=3, p=0.049). Q group spent a mean 1.64% of time feeding on *C. peltata*, while groups A, B and N fed on *C. peltata* for a mean 12.37%, 16.32% and 14.08 % of time respectively. (Fig.9). Only two of the four individuals from Q group were seen to ingest *C. peltata* over the study period, both for a small amount of time (0.35% and 6.22% of total time spent feeding).

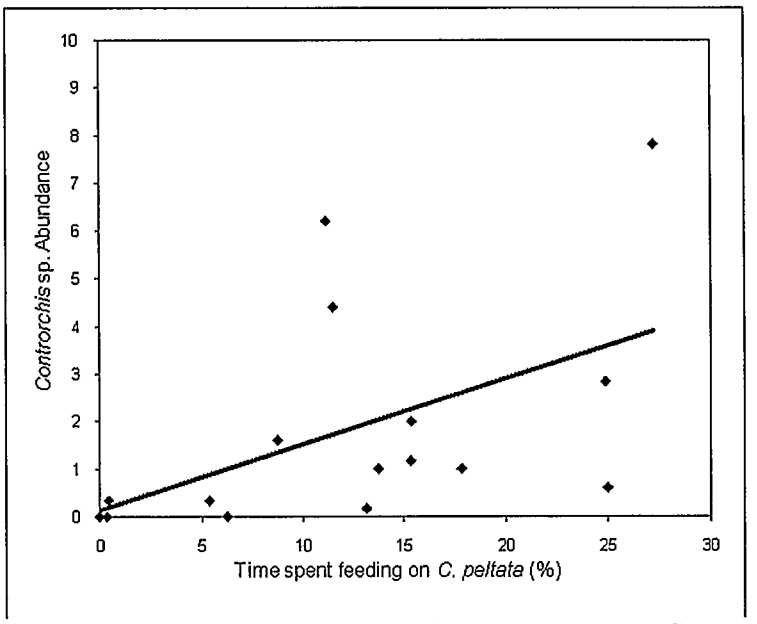


Figure 8: *Controrchis* sp. Abundance vs. Time Spent Feeding on *Cecropia peltata*
Controrchis sp. Abundance (number of eggs / gram of positive and negative fecal samples) vs. amount of time spent feeding on *Cecropia peltata* by individual (*n* =17)

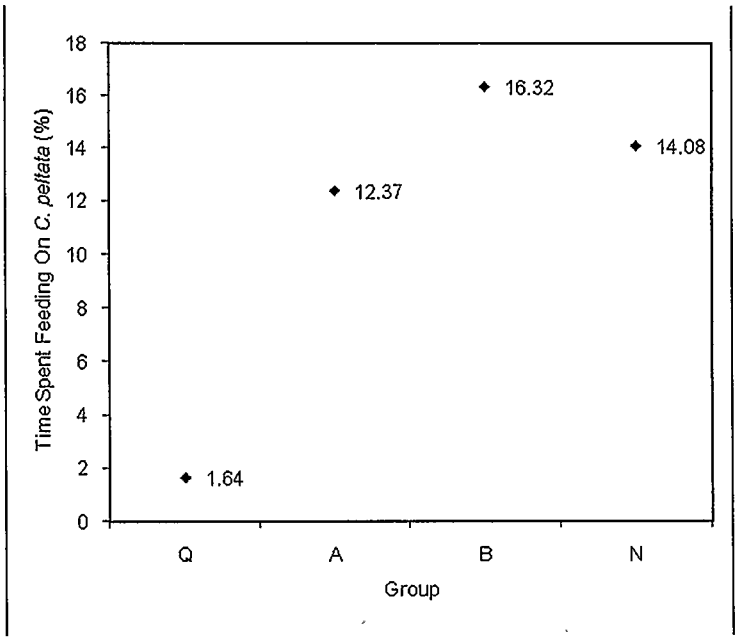


Figure 9: Mean Percentage of Time Spent Feeding on *Cecropia peltata* by Group

Mean percentage of time spent feeding on *Cecropia peltata* by group. 17 individuals (Q = 4; A = 3; N = 5; B = 5); 2.33 total hours spent feeding on *C. peltata* (Q = 5.00 min; A = 38.22 min; B = 61.45 min; N = 35.05 min)

Parasite Avoidance Strategies

It has been suggested that *Alouatta* sp. travel down in the canopy prior to defecating in order to avoid contaminating foliage with fecal parasites. To examine the possibility that defecation from low in the canopy serves as a parasite avoidance strategy, I investigated whether the canopy height of groups during defecation was related to the average prevalence of directly transmitted parasites (*G. duodenalis*, *Trichostrongylid* sp.). Although *Trichuris* sp. is a directly transmitted parasite, as only six eggs were recovered, it was excluded from analysis examining parasitism and defecation height.

Fifty-seven defecation events were observed during this study (Q group=21, A group=11, B group=15, N group=10). 68.42% involved a decrease in canopy height

before defecation and there were no instances of a group moving higher in the canopy to defecate. (Fig.10). Data concerning changes in canopy height were not available for 8.77% of defecation events due to factors such as weather or group members being obscured by vegetation. Information pertaining to the canopy height of groups after defecation was available for 52 defecation events (Q group=19, A group=11, B group =13, N group=9). An increase in canopy height after defecation was observed to occur in 73.08% of instances and groups were never observed to decrease in canopy height after defecating (Fig.11).

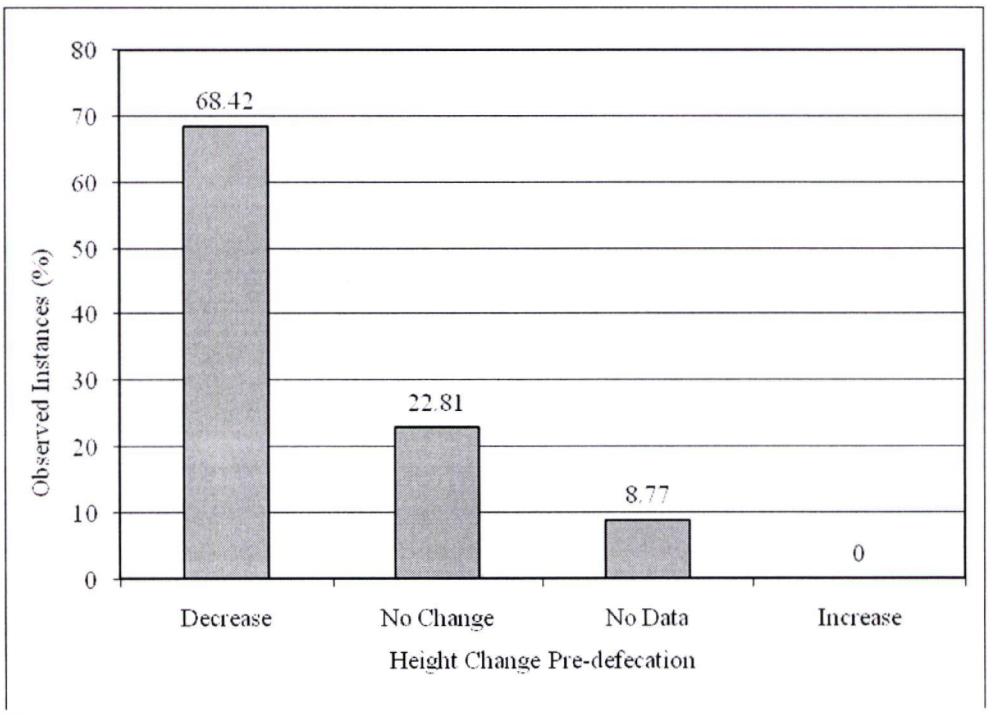


Figure 10: Changes in canopy height pre-defecation

Changes in canopy height prior to defecation observed during group defecation events (n = 57 instances)

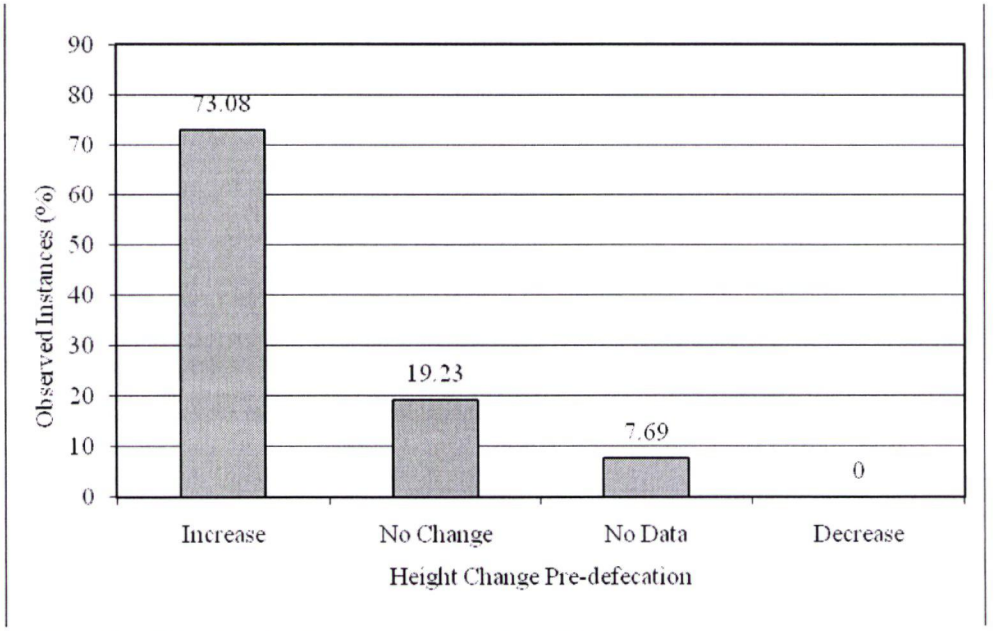


Figure 11: Changes in canopy height post-defecation

Changes in canopy height after defecation observed during group defecation events (n = 52 instances)

Groups N (90.00%) and Q (76.19%) came down in the canopy most consistently to defecate, while a decrease in canopy height pre-defecation was observed from groups A and B in 63.64% and 53.33% of instances respectively (Fig.12). Groups B and A were observed to travel up in the canopy after defecating in the most instances (91.67% and 81.82% respectively) (Fig.13). Groups A and B did not decrease in canopy height before defecating in as many instances as did groups Q and N, yet they came up in the canopy after defecating more times than did groups Q and N.

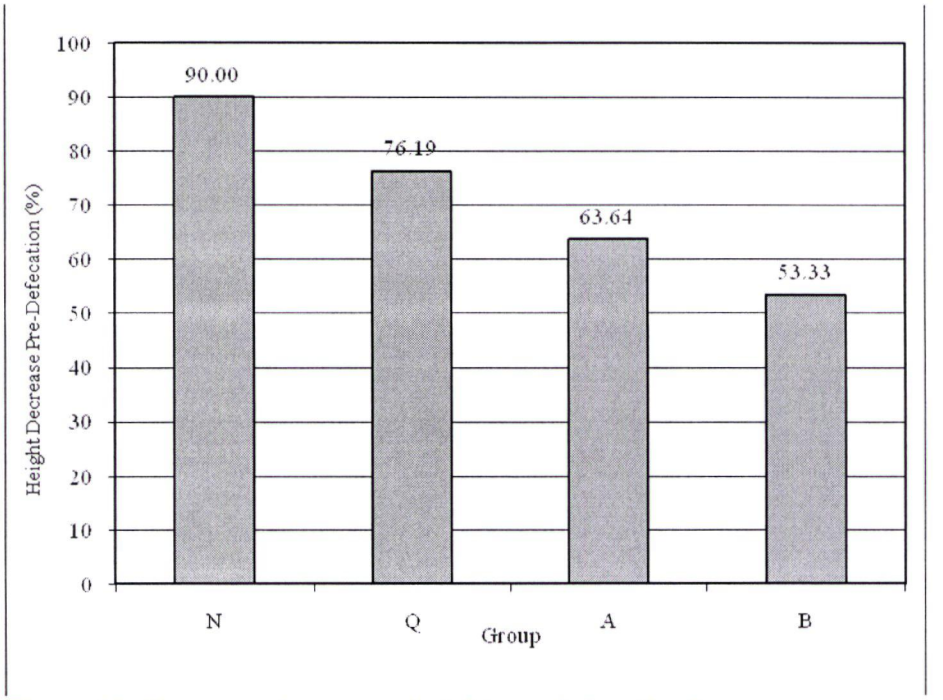


Figure 12: Decreases in canopy height pre-defecation by group

Percentage of observed instances in which study groups traveled down in the canopy before defecating (Q = 21; A group = 11; B = 15; N = 10)

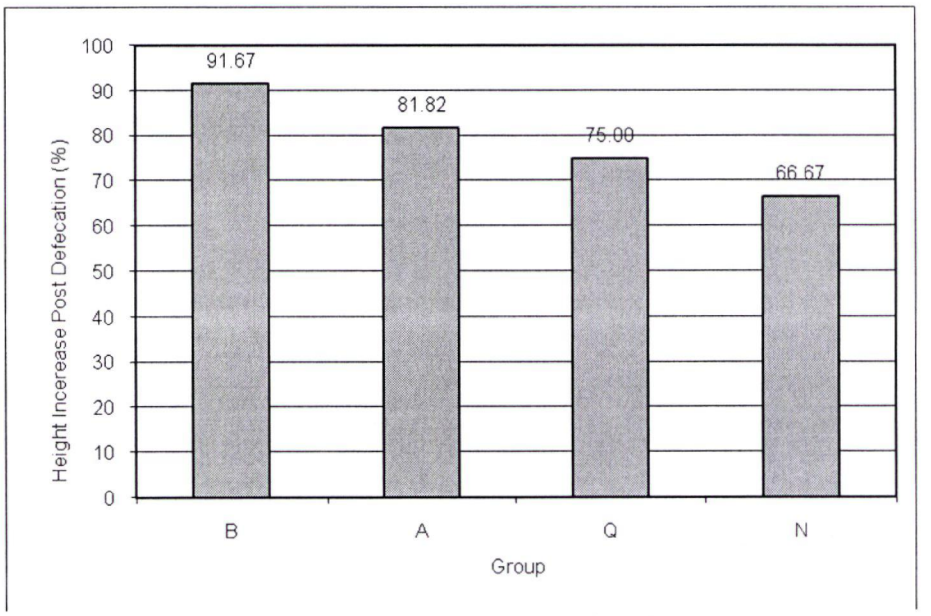


Figure 13: Increases in canopy height post-defecation by group

Percentage of observed instances in which study groups travelled up in the canopy after defecating (Q = 19; A = 11; B = 13; N = 9)

When the heights recorded during defecation events for each group were re-coded (1 = 0-5m, 2 = 6-10m, 3 = 11=15m, 4 =16=20m), the average height of defecation was quite similar among the groups, (~11-15m) (Fig.14). While all groups defecate from approximately the same level in the canopy, it appears that groups that defecate from this canopy level (~11-15m) more consistently may be at a lower risk for infection with directly transmitted parasites.

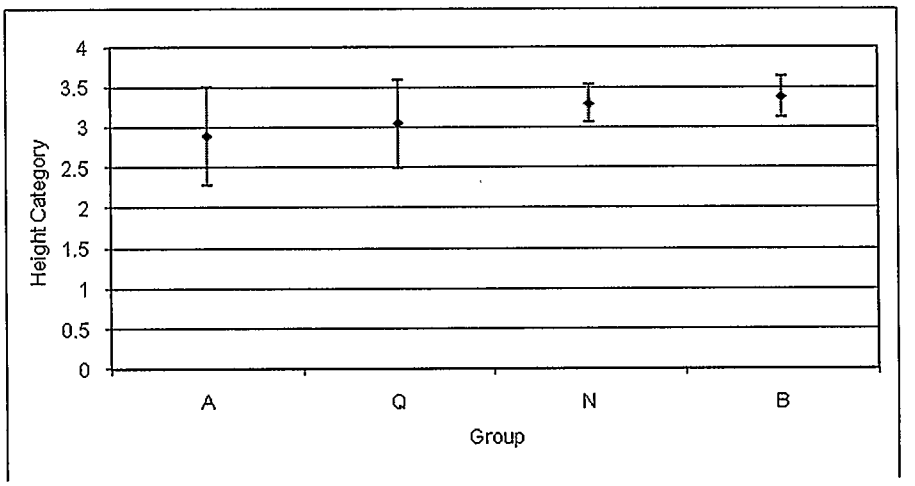


Figure 14: Average Defecation Height by Group

Average canopy height of study groups during observed instances of defecation recoded into categories 1 - 4 (1 = 0-5m, 2 = 6-10m, 3 = 11=15m, 4 =16=20m)

The lowest prevalence of *G. duodenalis* and *Trichostrongylid* sp. were recovered from the groups that defecated from low in the canopy most consistently (Q and N) (Table 10). Conversely, a higher prevalence of *G. duodenalis* and *Trichostrongylid* sp. were recovered from the research groups that were observed to defecate from high in the canopy in more instances (A and B) (Fig. 15). Although these groups moved up in the canopy post-defecation in the most instances, this behavior does not appear to be as

efficient a strategy to avoid contamination with fecal parasites as is defecating from ~11-15m consistently.

Table 10: Defecation Behaviour and Sample Prevalence by Group

Measure	Group			
	A	B	N	Q
# Individuals	3	5	5	4
# Fecal Samples	17	28	24	24
Height Decrease Pre-Defecation (%)	63.64	53.33	90.00	76.19
Height Increase Post-Defecation (%)	81.82	91.67	66.67	75.00
Giardia duodenalis Prevalence (%)	58.82	67.86	54.16	0.00
Trichostrongylid sp. Prevalence (%)	29.41	28.57	4.16	20.83

Height decrease pre-defecation: percentage of observed instances in which groups travelled down in the canopy before defecating (Q = 21; A group = 11; B = 15; N = 10); Increase in canopy height post-defecation: percentage of observed instances in which groups travelled up in the canopy after defecating (Q = 19; A = 11; B = 13; N = 9). Sample prevalence (fecal samples testing positive / fecal samples analyzed)

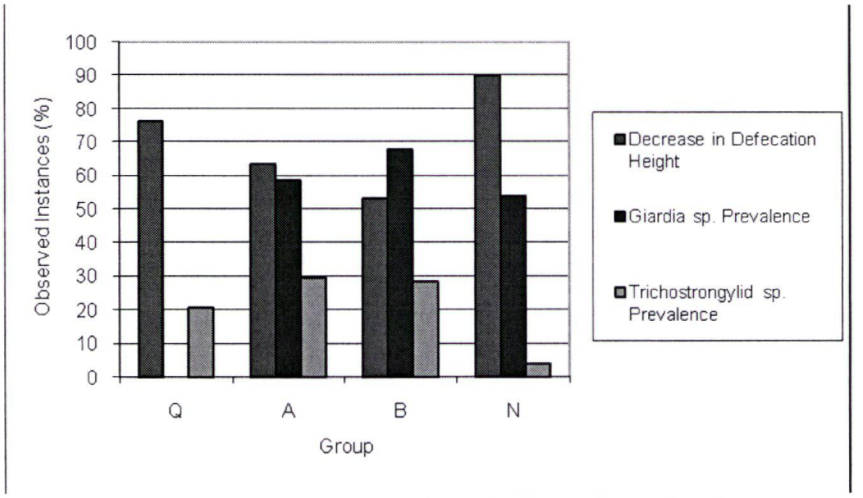


Figure 15: Decrease in Defecation Height vs. Sample Prevalence by Group

Percentage of instances in which a decrease in canopy height was observed pre-defecation vs. the sample prevalence (fecal samples testing positive / fecal samples analyzed) of *Giardia duodenalis* and *Trichostrongylid* sp. for each study group

Results Summary

- The parasites present in the study population included: *Controrchis* sp., *Giardia duodenalis* (Assemblage B), *Trichuris* sp. and *Trichostrongylid* sp. Overall, infection levels were relatively low.
- A significantly higher prevalence of *Controrchis* sp. was recovered from adults relative to juveniles and there were no differences in parasite prevalence between males and females.
- There was significant variation in *Controrchis* sp. prevalence and *Giardia* sp. prevalence among groups.
- The abundance of infection with a given parasite species did not affect the amount of time individuals spent inactive or feeding.
- The prevalence of infection with a given parasite did not differ by season or between study and tourist monkeys
- The consumption of ants contained on *Cecropia peltata* was identified as a possible source of *Controrchis* sp. Individuals that time spent more time feeding on *Cecropia peltata* had significantly more abundant *Controrchis* sp. infections. The group from which no *Controrchis* sp. was found fed on *C. peltata* for significantly less time than did other groups.
- Groups tended to defecate at a height of 11-15m and never ascended the canopy before defecating. A decrease in canopy height before defecation was observed in 68% of defecation events, and groups moved up in the canopy after defecating in 73% of defecation events.

- Groups that descended in the canopy more consistently before defecating had a lower prevalence of directly transmitted parasites.

CHAPTER 5: DISCUSSION

Primatological research over the past three decades has determined that the social and mating systems of primates are influenced by ecological factors such as the availability of certain foods and the risk of predation (Wrangham, 1980; Sterk et al. 1997; Snaith and Chapman, 2007). Recently, there has been an increase in research examining infectious disease as a selective force impacting social organization and behaviour in alloprimates. Evidence of infectious disease causing mortality in non-human primates is abundant and much of this research has focused on mortality as a result of epidemics (Pope, 1995; Carpenter, 1965; Bermejo et al. 2006) or ectoparasitic infections (Milton, 1996). The effects of parasitic infection on the health and behaviour of primates is not well understood and there are large geographical sampling gaps that impede our current knowledge of parasitic infections in wild primates (Nunn and Altizer, 2006; Hopkins and Nunn, 2007). Although research has examined the intestinal parasites of *A. belzebul*, *A. caraya* and *A. palliata*, there are few accounts of parasitic infections in *A. pigra* (Stuart et al. 1998; Vitazkova and Wade, 2006; Eckert et. al, 2006, Trejo-Macias et al. 2007).

Parasites Recovered

One microparasite and three species of macroparasite were recovered. Of the macroparasites, only one has an indirect lifecycle. *Controrchis* sp. is a digenean trematode and requires two intermediate hosts, a mollusk and an arthropod, to complete its lifecycle (Eckert et al. 2006; Vitazkova and Wade, 2007). *Controrchis* sp. is a fluke of the gallbladder and it is suspected that the species of *Controrchis* recovered in this study is *C. biliophilus*, however identification is tentative as no adult larvae were recovered.

Evidence supporting *Controrchis* species identification came from an unpublished report of *C. biliophilus* in *A. pigra* from Mexico (Bonilla-Moheno 2002), identification of adult *C. biliophilus* during necropsies of *A. palliata* from Costa Rica (Stuart et al. 1990), and the recent recovery of eggs suspected as *C. biliophilus* from *A. pigra* in Belize and Mexico of a similar size and morphology as those recovered in this study (Vitazkova and Wade, 2007).

One species of *Trichostrongylid* was recovered. Trichostrongyles are a diverse set of nematodes that have been reported from all vertebrates except fish (Noble et al. 1989). Infections are common in ruminants such as cattle and adult parasites occur mainly in the gastrointestinal tract of the definitive host. Eggs are passed out in feces and rhabditiform larvae feed on fecal microflora until development into the third larval stage (L3), which attach to plants and are infective to definitive hosts (Roberts and Janovy, 2005). Although the Trichostrongyle recovered in this study was not identified to the species level, it is assumed that the lifecycle is not unlike that of other Trichostrongyles. Many strongyle-type nematodes have been recovered from populations of *Alouatta*, yet few have been positively identified to the species level (Stuart et al. 1998, Stoner and Di Piero, 2006; Eckert et al. 2006; Trejo-Macias et al. 2007). This is currently the second account of *Trichostrongylid* sp. in a population of *A. pigra* (Stoner and Di Pierro, 2006) and this parasite has not been reported in other Neotropical primates.

Trichuris sp. eggs were only recovered from three fecal samples and identification to the species level was not possible. *Trichuris* sp. are commonly known as whipworms and are transmitted through the fecal-oral route. This parasite must undergo several larval molts after deposition in the feces before becoming infective to the definitive host; eggs

are typically acquired from contaminated soil and adults develop in the large intestine of the definitive host (Roberts and Janovy, 2005). This is the first study to report the presence of *Trichuris* sp. eggs in *A. pigra*.

Giardia duodenalis was the only microparasite recovered. *Giardia* sp. are protozoan parasites of the digestive tract and are directly transmitted through the ingestion of infective cysts in the feces (Roberts and Janovy, 2005). *Giardia* sp. has been identified in *Alouatta palliata* (Stuart et al. 1998; Stoner, 1996) and there is one unpublished (Bonilla-Moheno, 2002), and one recently published report of *Giardia* sp. in *A. pigra* (Vitazkova and Wade, 2007). Molecular testing of *Giardia* sp. cysts recovered from *A. pigra* populations in Belize have been confirmed as *Giardia duodenalis* (syn. *G. lamblia, G. intestinalis*) Assemblages A and B (Vitazkova and Wade, 2007). Molecular testing performed during this study determined that the population of *A. pigra* at Monkey River is infected with *G. duodenalis* (syn. *G. lamblia, G. intestinalis*) Assemblage B.

Giardia sp. is a parasite that has been found in many animals including mammals, amphibians and birds. *G. duodenalis* Assemblage B is one of the most widespread *G. duodenalis* subtypes, having been reported in humans, other primates, beavers and rats (Thompson, 2000; 2004). Although *G. duodenalis* Assemblage B has a wide host range, this subtype has not been isolated from livestock (Thompson, 2000), suggesting an alternate source of infection for the animals in this study.

It is interesting to note the absence of *Trypanoxyuris* sp. in this population of *A. pigra*, which has experienced drastic ecosystem disturbance in the last decade. Vitazkova and Wade (2007) suggest that pinworms are a natural part of *A. pigra*'s gastrointestinal fauna and that the absence of this parasite may be an indicator of ecosystem disturbance

(Vitazkova and Wade, 2007). The study site at Monkey River was in the direct path of Hurricane Iris in 2001, experiencing a population reduction of 88% (Pavelka et al. 2007) and a 35% loss of major food trees (Behie and Pavelka, 2005).

Four species of endoparasite, all occurring at a relatively low abundance and prevalence, were recovered in this study. The low diversity and number of parasite species detected are not unusual for *A. pigra*. Previous research of *A. pigra* has recovered similar numbers of macroparasites (Trejo-Macias et al. 2007 [n=4] Stoner and Di Pierro, 2006 [n=4]; Eckert et al. 2006 [n=4], Vitazkova and Wade, 2006 [n=2]) and comparable numbers of microparasites (Stoner and Gonzales Di Pierro, 2006 [n=4]; Eckert et al. 2006 [n=2], Vitazkova and Wade, 2006 [n=2]), Trejo-Macias et al. 2007 [n=1]). With the exception of *Trichuris* sp. all the endoparasites identified in this study have been detected in other populations of *A. pigra* (see Table 1).

Variation in Parasitism by Age and Sex

Parasite prevalence did not vary between males and females for any of the parasite species recovered and these results are consistent with other published accounts of endoparasitism in *A. pigra* (Eckert et al. 2006; Vitazkova and Wade, 2006). However, a higher prevalence of *Controrchis* sp. was found in adults relative to juveniles. Previous research is in agreement with these findings (Eckert et al. 2006, Vitazkova and Wade, 2007) although the results from this study may have been influenced small sample sizes or the fact that adults were over-represented (n=11) relative to juveniles (n=6).

Variation in Parasitism by Group

Of the parasite species recovered, *Controrchis* sp. showed the strongest association with group membership, a finding in agreement with Vitazkova and Wade (2007). There were substantial differences in *Controrchis* sp. prevalence among study groups; the highest prevalence was recovered from N group, while no *Controrchis* sp. was present in Q group. Although the reason for this variation is not completely clear, it may be related to differences in home range size. A larger home range may result in contact with a greater number of potential infective parasitic stages (arthropods) in the environment (Nunn et al. 2003a; Vitone et al. 2004). N group ranged over the largest area during this study (3.76 ha), while Q group had the smallest home range (2.64 ha). Significant differences in *Controrchis* sp. prevalence may also be tied to diet; this is discussed further in the section entitled "*Controrchis* sp. Abundance and the Ingestion of *Cecropia peltata*".

The average sample prevalence of *G. duodenalis* varied significantly among research groups; infection levels were similar among three groups, yet no *G. duodenalis* was detected in Q group. The comparative absence of this parasite in Q group is puzzling given that *G. duodenalis* is directly transmitted and infections do exist in this monkey population. Inter-group variation in protozoan (*Blastocystis* sp., *Entamoeba coli*, *Giardia* sp.) infection levels have been documented from other populations of *A. pigra* (Stoner and Di Pierro, 2006, Vitazkova and Wade, 2007). Although factors such as group size, proximity to humans and / or the presence of *Ateles geoffroyi* have all been suggested as factors that may account for variation in protozoan infection levels, Q group did not contain fewer monkeys than did other groups and due to their location (nearest to a

human settlement), these monkeys would potentially have a greater probability of coming into contact with humans than would other groups.

Parasitism and Behaviour

Infection with intestinal parasites such as *Giardia* sp. and various nematodes are known to be detrimental to the system of the host (Huffman, 1997; Olson and Buret, 2001; Roberts and Janovy, 2005). *Giardia* sp. infections may result in decreased absorption of water, maldigestion, and atrophy of the villus lining the GI tract (Olson and Buret, 2001), while infection with other intestinal parasites such as *Trichuris* sp. can cause anemia and dysentery (Roberts and Janovy, 2005).

The effect of intestinal parasites on behavior, fitness and mortality in non-human primates is not well documented (Stoner and DiPierro, 2006; Nunn and Altizer, 2006; Huffman, 1997). Although endoparasitic infections may result in a variety of symptoms such as malaise and a change in appetite, outward signs of disease may be difficult to observe and document (Hart, 1990); this may be responsible for the lack of research that has explored the relationship between parasitosis, behavior and fitness. In this study, no relationship was detected between activity levels or time spent feeding and the abundance of macroparasites or the prevalence of *Giardia duodenalis*. The lack of an association between parasite infection levels and behavior in the study population may be explained by the relatively low species diversity, prevalence and abundance of gastrointestinal parasites. Only four parasite species were recovered, the prevalence of each was <50%, and only one parasite species occurred at an abundance of >1 eggs/gram. It is possible that the animals in the study population are experiencing internal pathologies related to

parasitic infection, with few observable outward symptoms such as decreased levels of activity or feeding. However, the low diversity of endoparasites recovered from the study population, in association with the lack of a relationship among parasitosis, time spent feeding and activity levels may also be interpreted to mean that these monkeys are relatively healthy in terms of gastrointestinal parasitic infections.

Parasitism and Seasonality

Moist conditions are known to be conducive to the proliferation of many parasite species (Roberts and Janovy, 2005). However, parasite prevalence did not vary between the wet and dry months for any of the parasites recovered in this study. Huffman et al. (1997) found no seasonal difference in *Trichuris* sp. prevalence in a population of *Pan troglodytes* in the Mahale Mountains; *Trichuris* sp. eggs are very thick walled and highly resistant to environmental conditions, which may explain why this parasite does not tend to vary between wet and dry seasons (Roberts and Janovy, 2005). The lack of seasonal variation in *Controrchis* sp. prevalence parallels the results of Vitazkova and Wade (2007) who suggest that the intermediate hosts required for transmission of this parasite may not be impacted by changes in rainfall or moisture in such a way that ingestion by the monkeys differs between the seasons. A lack of seasonal variation in *G. duodenalis* prevalence among *A. pigra* has been reported elsewhere (Vitazkova and Wade, 2006).

Parasitism and Human Contact

Humans and alloprimates are susceptible to many of the same diseases and zoonotic disease transmission has been documented from a number of wild primate groups (Muller-Graf et al. 1997; Grazyk et al. 2002). Contact with humans and visitation by tourist groups are thought to be responsible for the transmission of *Giardia* sp. to populations of both *Gorilla gorilla beringei* in Uganda (Grazyk et al. 2002) and *A. pigra* in Belize (Vitazkova and Wade, 2007). However, this study found no relationship between monkeys experiencing high and low levels of human contact and the prevalence of the parasites recovered.

Vitazkova and Wade (2007) recovered the highest prevalence of *G. duodenalis* from groups of *A. pigra* living near human settlements, although it was not possible to isolate the effects of human presence on the prevalence of *G. duodenalis* infections. It is interesting to note that all individuals infected with *G. duodenalis* in the aforementioned study are known to either commonly descend to the ground, accept food from tourists (Community Baboon Sanctuary), or experience contact with humans and human feces through archaeological work being performed within the home ranges of monkey groups (Calakmul Biosphere Reserve); no *Giardia* sp. was recovered from groups that were relatively isolated from human contact (Cockscomb Basin Wildlife Sanctuary).

The lack of variation in the prevalence of parasites between monkeys experiencing high and low levels of human contact may be due to sampling error. While I collected multiple fecal samples from known study individuals, I was only able to obtain one fecal sample / individual from tourist monkeys. Alternatively, as the monkeys in Monkey River rarely descend to the ground and are not hand fed by tourists, high levels

of human visitation may not automatically result in the type of contact necessary for the increased transmission of directly transmitted parasites in arboreal primates.

Controrchis sp. Abundance and Ingestion of *Cecropia peltata*

This study documented the presence of *Controrchis* sp. (Dicrocoeliidae) in the study population. *A. pigra* are typically folivorous and frugivorous and all dicrocoelid trematodes have indirect lifecycles and require intermediate hosts for successful completion of the lifecycle. Snails are suspected as the first intermediate host in the lifecycle of *Controrchis* sp. and Vitazkova (2005) first made the suggestion that monkeys may acquire *Controrchis* sp. from the ingestion of metacercariae in the second intermediate host (*Azteca* sp. ants). To further examine this hypothesis, I investigated the source of infection with *Controrchis* sp. as ants ingested by monkeys when feeding on a tree species (*Cecropia peltata*) that has a mutualistic relationship with *Azteca* sp. As expected, this study found a positive relationship between the percent of time spent feeding on *C. peltata* and the abundance of *Controrchis* sp.; individuals that spent more time feeding on *C. peltata* had significantly more abundant *Controrchis* sp. infections.

Cecropia spp. occurs throughout the neotropics and constitutes a large part of the diet of A. *pigra* (Silver et al. 2000; 1998; Horwich and Lyons, 1990). This tree species is commonly found in cleared areas, along roadsides and in secondary growth forests. *Cecropia peltata* is a myrmecophyte- a type of plant that lives in association with a colony of ants and possesses specialized organs in which the ants live. In Belize, *C. peltata* has a mutualistic relationship with *Azteca* sp.; ants protect the trees from foreign invaders such as vines and herbivores, while residing inside the trunks of trees and

feeding on Mullerian bodies on leaf petioles and pearl bodies on the underside of leaves (Longino, 1989; Horwich and Lyons, 1990; Rickson, 1977). When an animal disturbs the tree, the ants emerge from the trunk and branches to attack the intruder. Volatile plant cues, induced through leaf damage by herbivores, cause *Azteca* spp. to recruit other ants to the site of damage (Agrawal and Dubin-Thaler, 1999) and the occupation rate of *Cecropia* sp. by *Azteca* sp. is typically very high (e.g. 84% occupancy rate) (Logino, 1989).

A. pigra can cause extensive damage to the leaves of *C. peltata* when feeding and it is suspected that monkeys ingest *Azteca* sp. infected with *Controrchis* sp. when feeding on *C. peltata*. Although no metacercariae have been recovered from ants to date (Vitazkova, 2005), intermediate life stages (cercariae) of *Controrchis biliophilus* have been recovered from snails in Belize (S. Vitazkova, pers. comm.). In conjunction with the aforementioned data, the positive relationship found between *Controrchis* sp. abundance and the amount of time spent feeding on *C. peltata* by individuals in this study suggests that *A. pigra* become infected with *Controrchis* sp. through the ingestion of ants infected with metacercariae when feeding on *C. peltata*.

There were significant differences in the amount of time spent feeding on *C. peltata* among groups; no *Controrchis* sp. was recovered from Q group, which ate significantly less *C. peltata* than did the other study groups. Monkey River experienced a Category 4 hurricane in 2001, which defoliated the majority of the area and there is currently a high density of *C. peltata* growing within the research site. Although no vegetation transects were performed during this study, it is suspected that the high burden of *Controrchis* sp. recovered from N group and the absence of *Controrchis* sp. in Q group

may be due to a disproportionate density of *C. peltata* (and infective arthropods) occurring within the ranges of these monkey groups. Subjectively, there is a much lower density of *C. peltata* in the range of Q group and this group fed on *C. peltata* for significantly less time than did other groups.

Vitazkova and Wade (2007) report a 67% prevalence of *Controrchis biliophilus* from *A. pigra* in continuous forests and a 95% prevalence from groups in fragmented forests, further suggesting that factors of the environment may be responsible for the clustering of *Controrchis* sp. in certain groups. As *Cecropia* sp. tend to grow in areas of ecological disturbance, the high prevalence of *Controrchis* sp. reported to occur in fragmented forests is to be expected if infection occurs through the accidental consumption of arthropods contained on these trees.

Parasite Avoidance Strategies

Selective defecation, in the form of defecating from low in the canopy in areas of sparse understorey has been observed in a number of *Alouatta* species (Kowalewski and Zunino, 2005; Gilbert, 1997; Henry and Winkler, 2001). Past research speculates that this behaviour serves as a parasite avoidance strategy for folivorous primate species, decreasing the chance that foliage will become contaminated with fecal matter containing directly transmitted parasitic organisms. This study assessed the relationship between levels of directly transmitted parasites and defecation from low in the canopy.

Defecation in groups of *Alouatta* tends to be a synchronous event and I predicted that in order to decrease contact with infective stage parasites contained in fecal matter, a decrease in canopy height before defecation would be observed from research groups. In

almost 70% of defecation events, groups descended in the canopy before commencing defecation and no group ever traveled up in the canopy before defecating. Groups N and Q decreased in canopy height before defecating in the highest number of instances (90.00% and 76.19% respectively) and the prevalence of both *Giardia* sp. and *Trichostrongylid* sp. were the lowest in these two groups. In conjunction with the information gathered from defecation events, the low species diversity of directly-transmitted parasites recovered may suggest that defecation from low in the canopy is an adaptive behavior that helps animals avoid contact with directly-transmitted fecal parasites.

Summary

This is the first study of parasitic infection in the Monkey River population of *A. pigra*. Four species of gastrointestinal parasite were recovered, all with a relatively low overall prevalence and abundance. Although few *Trichuris* sp. eggs were found, this is the first account of *Trichuris* sp. in *A. pigra*. Results from this study provide circumstantial evidence as to the route of *Controrchis* sp. transmission (a parasite requiring an arthropod host) to a folivorous and frugivorous species of monkey through the accidental ingestion of ants when feeding on a myrmecophyte (*C. peltata*). In almost 70% of instances groups descended in the canopy before defecating and an increase in canopy height prior to defecation was never observed. Groups that descended in the canopy prior to defecating in the most instances had lower levels of directly transmitted parasites. In conjunction with low parasite infection levels and the few species of

parasites recovered, these results may suggest that defecation from low in the canopy is an adaptation to avoid endoparasitism that is effective.

Limitations of this Study and Directions for Future Research

The results from this study lend insight into how endoparasitic infections in the population of *A. pigra* in Monkey River relate to diet, activity patterns and certain aspects of the environment. As this research took place over a period of six months, the results reported represent a "snapshot" of parasitism in this population. This is a problem inherent in short-term studies, as such research does not reflect the dynamic relationship between hosts and parasites (Stuart et al. 1998). The number of individuals observed in this study resulted in a small overall sample size ($n = 17$), which may have decreased the ability to detect differences in parasite levels between age classes or sexes. Although multiple fecal samples were collected from known individuals to estimate levels of parasitism, it may be necessary to perform necropsies on dead animals, or sample the perianal region of captured hosts for a representative estimate of the prevalence of parasitic infection (Stuart et al. 1990). Also, as fecal floats in Sheather's solution and the use of Aqua-Glo testing kits were the only recovery methods used to extract parasites from fecal samples, there remains a possibility that this study did not recover all species of endoparasite present in the study population. Initially, feces were preserved in a formalin solution that led to the desiccation of a number of fecal samples that had to be discarded. This resulted in the fact that in some cases, fecal samples from an individual were not available for each month of study. The lack of fecal samples from tourist-visited monkeys limited the comparison that could be made between levels of parasitism in

tourist and study monkeys. A future study should attempt to collect fecal samples from known individuals in the tourist monkey population to obtain a more comprehensive understanding of how parasitism may differ between monkeys that experience different levels of human contact.

There is a need for longitudinal research documenting the presence and impact of parasitic infections in populations of *A. pigra*, as well as the effect that ecological factors have on parasitic infections. Results from this study suggest that *A. pigra* become infected with *Controrchis* sp. through the ingestion of *Azteca* spp. when feeding on *C. peltata*. Confirmation of this relationship will depend upon the future collection of mollusks and arthropods containing the intermediate stages of *Controrchis* sp.

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APPENDIX A: DATA COLLECTION SHEETS

Ad Libitum Fecal Event Sheet

Researcher ID:_____ **Date (d/m/y):**_____ **Time:**_____

Group:_____ **Group Composition:** _____

Group Activity: I T F S O **Group spread (beg):** <2 2-5 5-10 >10

Location (tree tag #): _____ **Tree Species:** _____ **Canopy Height:** L / M / H

Tree Crown Size: S / M / L **Weather:** R / PC / C / S **Windy:** Y / N **Temp (c):** ____

1st individual to move: _____ **Distance from ground b/f Defecation(m):** _____

Time and ID of 1st defecation: _____ **Group spread @ 1st defecation:** _____

Time and ID of last defecation: _____ **Group spread @ last defecation:** _____

Distance from Ground At Defecation (m): _____

Distance from Ground Post-Defecation (m):_____

Additional notes on defecation event:

_____

_____

_____

_____

_____

_____

_____

APPENDIX B: PARASITE RECOVERY DATA SHEETS

Ind.	Demographic		Giardia Cysts (Presence/Absence)						Proportion Samples Positive	Mean Percentage Infection (group)
	Group	Age/Sex	Jan	Feb	March	April	May	June		
1	Q	AM	N	N	N	N	N	N	0.00	0.00%
2	Q	AF	N	N	N	N	N	N	0.00	
3	Q	JF	N	N	N	N	N	N	0.00	
4	Q	JF	N	N	N	N	N	N	0.00	
5	A	AM	N	Y	Y	N	N	Y	0.50	60.00%
6	A	AF	Y	N	Y	Y	Y	/	0.80	
7	A	JF	Y	Y	N	Y	N	N	0.50	
8	B	AM	Y	Y	Y	Y	Y	/	1.00	68.67%
9	B	LF	N	N	Y	Y	Y	N	0.5	
10	B	AF	Y	Y	Y	N	/	N	0.6	
11	B	SM	Y	Y	N	Y	N	N	0.5	
12	B	JM	Y	Y	Y	Y	Y	N	0.83	
13	N	AM	Y	N	Y	N	/	N	0.40	49.33%
14	N	LF	Y	N	Y	N	/	Y	0.60	
15	N	SF	/	Y	N	Y	Y	Y	0.80	
16	N	JM	Y	Y	N	Y	N	Y	0.67	
17	N	JF	/	N	N	N	/	/	0	

Indiv	Group	Age/Sex	Trichostrongylid Intensity (Raw Count)						Eggs/Gram Total (mean)	Prop +ve	Group Intensity	Group Prevalence
			Jan	Feb	March	April	May	June				
1	Q	AM	1	0	4	0	0	0	0.83	0.33	0.375 eggs/gram	20.83%
2	Q	AF	1	0	2	0	1	0	0.66	0.50		
3	Q	JF	0	0	0	0	0	0	0.00	0.00		
4	Q	JF	0	0	0	0	0	0	0.00	0.00		
5	A	AM	0	2	0	0	1	0	0.50	0.33	0.471 eggs/gram	27.77%
6	A	AF	0	0	0	0	0	/	0.00	0.00		
7	A	JF	2	1	2	0	0	0	0.83	0.50		
8	B	AM	0	4	0	1	1	/	1.20	0.60	0.393 eggs/gram	30.00%
9	B	LF	1	0	1	0	0	0	0.33	0.33		
10	B	AF	1	0	1	0	/	0	0.40	0.40		
11	B	SM	0	0	0	0	0	0	0.00	0.00		
12	B	JM	0	1	0	0	0	0	0.16	0.16		
13	N	AM	0	0	0	0	/	0	0.00	0.00	0.083 eggs/gram	3.33%
14	N	LF	0	0	0	0	/	0	0.00	0.00		
15	N	SF	/	0	0	0	0	0	0.00	0.00		
16	N	JM	0	0	2	0	0	0	0.33	0.16		
17	N	JF	/	0	0	0	/	/	0.00	0.00		

Indiv	Group	Age/Sex	Trichuris Intensity (Raw Count)						Eggs/Gram Total (mean)	Prop +ve	Group Intensity	Group Prevalence
			Jan	Feb	March	April	May	June				
1	Q	AM	0	0	0	0	0	0	0.00	0.00	0.00 eggs/gram	0.00%
2	Q	AF	0	0	0	0	0	0	0.00	0.00		
3	Q	JF	0	0	0	0	0	0	0.00	0.00		
4	Q	JF	0	0	0	0	0	0	0.00	0.00		
5	A	AM	0	0	0	0	0	0	0.00	0.00	0.00 eggs/gram	0.00%
6	A	AF	0	0	0	0	0	0	0.00	0.00		
7	A	JF	0	0	0	0	0	0	0.00	0.00		
8	B	AM	0	0	0	0	0	/	0.00	0.00	0.036 eggs/gram	3.33%
9	B	LF	0	1	0	0	0	0	0.16	0.16		
10	B	AF	0	0	0	0	/	0	0.00	0.00		
11	B	SM	0	0	0	0	0	0	0.00	0.00		
12	B	JM	0	0	0	0	0	0	0.00	0.00		
13	N	AM	0	0	0	0	/	0	0.00	0.00	0.21 eggs/gram	10.66%
14	N	LF	0	0	0	0	/	0	0.00	0.00		
15	N	SF	/	0	1	0	0	0	0.20	0.20		
16	N	JM	0	0	2	0	0	2	0.67	0.33		
17	N	JF	/	0	0	0	/	/	0.00	0.00		

Indiv	Group	Age/Sex	Controrchis sp. Intensity (Raw Count)						Eggs/Gram Total (mean)	Prop +ve	Group Intensity	Group Prevalence
			Jan	Feb	March	April	May	June				
1	Q	AM	0	0	0	0	0	0	0.0	0.00	0.00 eggs/gram	0.00%
2	Q	AF	0	0	0	0	0	0	0.0	0.00		
3	Q	JF	0	0	0	0	0	0	0.0	0.00		
4	Q	JF	0	0	0	0	0	0	0.0	0.00		
5	A	AM	0	4	2	0	1	0	1.16	0.50	0.941 eggs/gram	48.88%
6	A	AF	1	0	4	1	2	/	1.60	0.80		
7	A	JF	0	1	0	0	0	0	0.16	0.16		
8	B	AM	0	1	1	1	0	/	0.60	0.60	1.178 eggs/gram	61.33%
9	B	LF	2	0	0	1	2	1	1.00	0.66		
10	B	AF	1	0	2	1	/	1	1.00	0.80		
11	B	SM	1	4	10	1	0	1	2.83	0.83		
12	B	JM	2	0	0	0	0	0	0.33	0.16		
13	N	AM	7	15	0	12	/	5	7.8	0.80	4.375 eggs/gram	71.80%
14	N	LF	5	7	6	0	/	4	4.4	0.80		
15	N	SF	/	8	1	7	9	6	6.2	1.00		
16	N	JM	0	5	1	4	2	0	2.0	0.66		
17	N	JF	/	0	1	0	/	/	0.33	0.33		