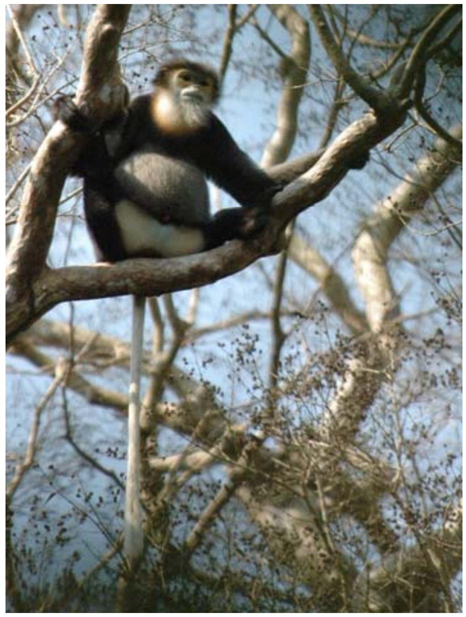
THE ECOLOGY AND CONSERVATION OF BLACK-SHANKED DOUCS (*PYGATHRIX NIGRIPES*) IN CAT TIEN NATIONAL PARK, VIETNAM



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The Ecology and Conservation of Black-shanked doucs (*Pygathrix nigripes*) in Cat Tien National Park, Vietnam

Thesis directed by Professor Herbert H. Covert

ABSTRACT

This dissertation addresses the nutritional ecology of the black-shanked douc (*Pygathrix nigripes*), an endangered colobine, in Cat Tien National Park, Vietnam. Because a lack of knowledge about the ecological needs and behavioral patterns of this colobine may well affect long-termed conservation planning, this study undertook a multi-year investigation of ranging behaviors and feeding choices in an effort to better understand the chemical make up of foods either selected or rejected by this species. In order to assess how selective the animals were in feeding, phenological transects were established to measure forest productivity throughout the year. These transects showed that Cat Tien NP is a highly seasonal environment with a marked wet season and dry season. This seasonality is a driving factor in where the doucs are ranging in order to track seasonal leaf resources. This study also showed selection for leaf material that was higher in protein, as well as higher in fiber, an interesting outcome in light of many arguments that lower fiber leaves will always be selected. Other important nutrients included higher levels of potassium, sodium, and iron. In addition to the feeding of these animals, an investigation into the parasites these animals harbor was undertaken to better understand the requirements and challenges they face. Parasite species recorded include *Strongyloides* sp., *Trichuris* sp., and *Physalopterra* sp., as well an unidentified tapeworm and pinworm. Eighty-three percent of the samples were infected by at least one species of parasite, and 58% of the samples were infected by at least two species. Black-shanked doucs were more likely to be infected by gastrointestinal parasites than to be free of infection ($p<.001$, $\chi^2=21.333$, df = 1). The results of this study can be applied to both in situ and ex situ conservation efforts. A better understanding of the habitat requirements of this species can aid in the preservation of landscapes for this animal, and a clearer knowledge of nutritional requirements can help in the care and feeding of these animals in captivity.

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CHAPTER 1: IF THESE ARE LEAF EATING MONKEYS AND THE FOREST IS MADE OF TREES WITH LEAVES, WHY BOTHER TO EVER MOVE?

1.1 Introduction

The purpose of this dissertation is to describe the environment, feeding selection, and parasite ecology of the black-shanked douc (*Pygathrix nigripes*) in Cat Tien National Park, Vietnam. In order to describe the behavioral ecology of the black-shanked douc, a number of topics are addressed that focus on the forest habitat of the doucs in Cat Tien National Park including: forest structure, forest phenology, soil chemistry, and weather data. Once the stage has been set, the ranging and feeding trees, nutritional chemistry, and parasite ecology of the doucs are discussed.

The black-shanked douc is a member of the Colobinae and is listed as endangered by the IUCN (Rawson et al., 2008). The black-shanked douc has been the focus of two dissertations to date: Dr. Hoang Minh Douc (2007) described the ecology and conservation status of doucs in two coastal protected areas in Vietnam and Dr. Ben Rawson (2009) described the socio-ecology of doucs in a national park in Eastern Cambodia. This study focuses on an additional aspect of the douc’s ecology through the study of the nutritional and parasitic ecologies. An understanding of the douc’s feeding ecology is an essential step towards developing detailed conservation plans for both this species and national park.

The black-shanked douc is likely an important indicator species for the health and status of Cat Tien’s forests, one of the largest and best-protected national parks in Vietnam. My study tests hypotheses regarding how the feeding ecology of the black-shanked douc varies across ecologically diverse habitats and yields an improved understanding of the habitat requirements for this species.

The dietary ecology of free ranging primates has been an exciting research topic from the 1960's to present. This research builds on existing scholarship and offers insights into the nutritional requirements of this species. Kirkpatrick (2011) provided the most comprehensive review of the behavioral ecology of Asian colobines to date, documenting interesting diversity in the diets of this radiation, and it is instructive to note that nothing on the black-shanked douc was included. While Nadler et al. (2003) reported that there had been no studies on this species, we now have information from both Vietnam and Cambodia (Hoang Minh Duc, 2007; Rawson, 2009).

This study provides additional information about this species in an environment that differs from those of previous studies. This research aims not only to add to our understanding about a primate species that is under-reported in the literature, but also to synthesize past scholarship with modern methodology and available tools for the conservation of threatened species. The use of nutritional tests on plant material taken from feeding observations, forest productivity measured through phenology transects, soil chemical sampling, and gastro-intestinal parasite measurements will increase our understanding of challenges doucs face in meeting their nutritional requirements.

This project yields data comparable to that collected on black-shanked doucs living in Nui Chua National Park, a coastal dry forest with an average rainfall of 697 mm, and Phuoc Binh National Park, a mountainous evergreen and conifer forest with an average rainfall of over 2000 mm, by Hoang Minh Duc (Hoang Minh Duc, 2007; Hoang Minh Duc et al., 2009) and the Seima Biodiversity Conservation Area, Mondulkiri Province, Cambodia, a wet inland forest with an annual average rainfall of 2,600 mm, by Ben Rawson (Rawson, 2006; 2009).

Ortmann et al. (2006) clearly noted the importance of feeding as a primary interaction of the primate with its environment and its influence on habitat systems, predator-prey interactions, and population dynamics. They also noted that diet can constrain species within landscapes and across seasons. Through the study of the feeding (behavior, nutrition, and selection) we can better understand the dietary requirements of black-shanked doucs. When this information is combined with Hoang Minh Duc's and Rawson's research, we can create effective and species-specific conservation action plans across its native range in Vietnam and into Cambodia. This study identifies some of the items that black-shanked doucs feed upon in Cat Tien, as well as examines some of the items they are rejecting to understand what drives this selection.

While a main goal of this research is the feeding behavior, we need to understand the dynamics of the forest; if we understand how the forest changes and what the species composition of the forest is, then we can begin to talk about habitat use and ranging behaviors of the animals. To understand the forest, three phenology transects were established within the Nam Cat Tien Sector in areas where the doucs were seen. These transects allowed a sample of the forest to be analyzed for tree height, DBH, basal area, and mortality. Once the initial measures were established, the phenology of the trees were measured: how many of each phase of leaf (young, mature, senescent) did the trees have, were there fruits present, were there flowers, did the trees lose their leaves? These data collected over the span of twenty-five months give a good indication of the seasonal variation in this forest. In addition, preliminary studies in the soil chemical content allow us to better understand how nutrients are cycling in the forest and again, how seasons vary.

When the forest structure has been described, and the variations in seasonal resource availability are known, then we can begin to address how the animals move about the landscape.

The phenology transects inform when there will be leaves in the forest, and as the chapter began, if they are 'leaf eating monkeys' then if the trees have lost all of their leaves, it would stand to reason that there would be variation in where the doucs are living and feeding. If we know where they are moving around and feeding- then we can begin to assess seasonal variation in ranging and resource tracking. An assessment of GPS data of douc encounters overlaid with feeding trees will qualify where they are spatially and temporally.

Once we know where they are and what they are feeding on, then we can look specifically at the chemical and nutritional composition of the leaves that are selected and the leaves that are not selected. These data allow for a better understanding of how they are meeting nutritional requirements and what variation do we see between Cat Tien NP and other areas where the doucs live. This may allow for addressing questions on dietary breadth or specialization of this species. Do the doucs adhere to models of selecting higher protein food items that also have lower levels of fiber, or are these not necessarily the motivating selection factors and doucs with their complex digestive systems, are undeterred by chemical or physical properties of many food items?

Moreover, we can look at another line of evidence that can speak to ranging, feeding, and health concerns, i.e. the monkey's parasite load. Parasites can run the gamut of being benign protagonists to causing serious health concerns or lead to the death of the host. This research samples the doucs to offer a preliminary look at the parasites they harbor. The forest block where these animals live is home to additional species of non-human primates that have their own behavioral traits that can lead to contact and infection with many species of parasites. The golden-cheeked gibbons (*Nomascus gabriellae*) are strictly arboreal, but still have parasites that spend a portion of their life cycle in the ground (Kenyon, 2007). All of these animals are using the same trees for feeding, locomotion, and possible sleeping sites; they are allowing arboreal creatures to have direct contact with other species of parasites because they use the same resources. Understanding what parasites they harbor can help explain dynamics in the community ecology of these sympatric species.

1.2 Research questions

Aim 1: To determine the effects of plant protein and fiber ratios on food selection.

Hypothesis 1: If black-shanked doucs are selective feeders, then they will actively select leaves that are higher in protein and lower in fiber.

Aim 2: To determine the effects of tannins on food selection.

Hypothesis 2: Black-shanked doucs will select foods that are lower in tannins.

Aim 3: To determine the effects of Cat Tien NP's seasonal environment on dietary habits of the black-shanked douc.

Hypothesis 3: Black-shanked doucs will show variations within their diets due to seasonal stress / availability.

Aim 4: To investigate the gastrointestinal parasites in black-shanked douc in Cat Tien NP.

Previous research (Kenyon, 2007) showed that black-shanked doucs did harbor some gastrointestinal parasites, but, unlike the golden-cheeked crested gibbons (*Nomascus gabriellae*), they did not harbor often-pathogenic parasites. While gibbons are not seen coming to the ground as doucs have been observed, a number of species of non-human primates do come into contact with one another in the forests of Cat Tien NP. I do not test any hypotheses regarding parasite infection during this research, as I do not have data regarding which individuals the feces were collected from. I can, however, make comparisons with previous research and present preliminary data on future research agendas dealing with parasitic infections.

1.3 Anticipations and outcomes

When I set out on this study, I anticipated that I would be able to habituate two or three separate groups of doucs within the study area. I followed the habituation methods discussed in Williamson and Feistner (2003), expecting habituation to take a few months; however, habituation never progressed beyond the animals fleeing when they noticed my presence. While I was unable to habituate any of the groups during this study, I was able to contact the doucs on most days. This contact allowed for collection of feeding behaviors, plant samples, fecal samples, and GPS data.

The forest structure of Cat Tien NP within the Nam Cat Tien Sector presented certain challenges to the habituation process. This area is relatively flat and the forest includes a complex understory, discontinuous middle story, and taller emergent trees. The animals were quite often alerted to my presence as I was encountering them. There were no vantage points available within this forest sector to observe the doucs without disturbing them.

Despite the unsuccessful habituation of the doucs, this study provides insight into the nutritional ecology and the gastrointestinal parasite ecology within this species. I also provide information on the forest structure and group seasonal ranging.

1.4 Goals of this research

This research provides a better understanding of the ecology of black-shanked douc in a natural setting. The goals of this research are three fold: the nutritional information can be used to provide adequate diets to animals in captivity, the ecological data can inform animal management specialists on the habitat requirements of the species, and finally a better understanding of parasitic infections can help understand how the doucs are coming into contact with other species or dealing with this energetic burden.

1.5 Outline of Dissertation

Chapter 1 provides an outline of the research aims and justifications for this work as well as some limiting factors of the study.

Chapter 2 details the historical background to this research. It provides information on the Colobinae, the genus *Pygathrix*, feeding ecology studies, an evaluation of the chemical environment, soil chemistry, Vietnam, Cat Tien NP, and conservation and threated species in Vietnam.

Chapter 3 covers the methods and materials used in this research. This includes information on transect preparation and monitoring, leaf material collection and preparation, plant chemical tests, soil analysis, and parasite collection methods.

Chapter 4 discusses the forest structure of Cat Tien NP. This includes data on the phenology transects, soil analysis, and weather data collected. Seasonal variation of habitat structure is discussed regarding the deciduous trees and alteration of the canopy throughout the year.

Chapter 5 provides information on the ranging of the animals and locations of feeding trees used by the animals established by GPS. This chapter also details the variation where the animals were found seasonally through the study.

Chapter 6 reports on the nutritional analysis of leaves ingested and rejected by black-shanked douc. This includes discussion about the tests conducted and the use and importance of each of the tested nutritional components of the leaves. Results of macro- and micro- nutrients, fiber, and tannins are evaluated.

Chapter 7 details the gastrointestinal parasites found in black-shanked doucs in Cat Tien NP. This includes parasite species identified and levels of infections.

Chapter 8 provides the discussion and conclusion of this dissertation and presents a brief summary of the key findings. This chapter also includes future research agendas in both feeding ecology and parasite ecology at multiple study sites.

1.5 Summary

The goal of this chapter was to summarize the dissertation and help introduce this research. The research aims and goals were listed along with outcomes and anticipations of this dissertation. Finally, the chapters of the dissertation were summarized.

CHAPTER 2: HISTORICAL BACKGROUND ON FEEDING ECOLOGY, PARASITE ECOLOGY, AND CONSERVATION IN VIETNAM

2.1 Introduction

In this historical background, I review the following topics that provide the intellectual context of this research: the subfamily Colobinae, feeding ecology, an evaluation of the chemical environment, parasitic infections, and conservation in Vietnam.

This research addresses many questions surrounding the feeding ecology of black-shanked doucs. Lambert (2011) noted that the study of an animal's feeding behavior is more than a matter of academic concern; it is also a priority of conservation work because food resources can be a limiting factor for populations. By better understanding the diet of an animal these food resources can be preserved in a wild setting or provided within a captive setting. Altmann and Wagner (1978) modeled optimal diets of primates based on a given food's availability and how much the animals should be eating. This perspective captures the importance of understanding an animal's ecology and how it makes feeding choices. When this research was first conceived, the black-shanked douc's feeding behavior was not only unpublished, it was unknown (Fleagle, 1999; Nadler et al., 2003). Kirkpatrick (2011) provided the most comprehensive review of the behavioral ecology of Asian colobines to date and nothing on the black-shanked douc was reported. This is no longer the case now that information about the dietary ecology of the black-shanked douc is available from two recent studies (Hoang Minh Duc et al., 2009; Rawson, 2009; Fleagle, 2013). The testing of the hypotheses posed by this research provides clarity to issues surrounding the dietary ecology of these animals. With this information, a conservation action plan for the species can be formulated for implementation in Cat Tien NP and across their range.

2.2 Colobinae

The Colobinae are a subfamily of the Cercopithecidae and can be characterized as a diverse radiation found throughout sub-Saharan Africa and south and southeast Asia (Oates and Davies, 1994). The colobines are distinguished from cercopithecines by a number of anatomical traits: deeper mandibles, enlarged salivary glands, the lack of cheek pouches, and complex stomach morphology (Oates and Davies, 1994; Fleagle, 1999). Mittermeier et al. (2013) identified 55 species of Asian colobines allocated to the genera *Trachypithecus* (20), *Pygathrix* (3), *Rhinopithecus* (5), *Nasalis* (1), *Presbytis* (17), *Simias* (1), and *Semnopithecus* (8); and 23 species of African colobines allocated to the genera *Colobus* (5), *Procolobus* (1), and *Piliocolobus* (17).

Colobines have been the focus of numerous studies both in Africa (e.g. Struhsaker, 1967; Oates, 1987; Oates et al., 1990; Chapman and Chapman, 2002; Fashing et al., 2007) and in Asia (e.g. Dolhinow, 1972; Oates et al., 1980; Davies et al., 1988; Kirkpatrick, 1998; Lippold and Vu Ngoc Thanh, 2008). While the species of Colobinae in Africa and Asia share many similarities, there are also interesting differences such as a wider range of body sizes in Asia, and while all African colobines are arboreal, a few of the Asian taxa are frequently observed on the ground including *Semnopithecus* in India and *Rhinopithecus* in China. In addition, a number of colobines in China and Vietnam occupy limestone forests. African colobines have a reduced thumb compared to that of Asian colobines (Szalay and Delson, 1979).

Kirkpatrick (2011) recently reviewed Asian colobines focusing on taxonomy, distribution, ecology, predation, reproduction, social behavior, and social organization highlighting both what is known about various species and also illustrates gaps within the field data. For feeding, the genus *Pygathrix* is unreported, the genus *Simias,* is also unreported, and the genus *Rhinopithecus* while reported, is incomplete. Fashing (2007) commented that, even though there has been a recent upsurge in ecological studies on colobines, many species have not been the focus of a single study.

Fashing (2011) recently reviewed the African radiation of colobines. He also focused on a broad scope of description covering ecology, predators, diet, ranging, activity patterns, food competition, biomass, reproduction, and social organization. Fashing (2011) also presented a wealth of information on group size, structure, and activity budgets for African colobines- very important information that is only now beginning to be elucidated for the Asian taxa and reveals the importance of gathering comparable data in Asia to add to our understanding of this radiation of primates.

2.3 Genus *Pygathrix*

Pygathrix sp. are found in Vietnam, Cambodia, and Laos (Fooden, 1996; Nadler et al., 2003). Presently, three species of *Pygathrix*, distinguished through coloration, geographic distributions, and genetics (Brandon-Jones, 1984; Groves, 2001; Roos and Nadler, 2001) are recognized: the red-shanked douc (*Pygathrix nemaeus*) (Linnaeus, 1771), grey-shanked douc (*Pygathrix cinerea*) (Nadler, 1997), and black-shanked douc (*Pygathrix nigripes*) (Milne-Edwards, 1871).

The doucs have been described as being primarily folivorous, but also have been noted to supplement their diet with other plant products. In field observations, Lippold (1998) estimated that 75% of the diet of her study sample consisted of small tender young leaves; “large tough” leaves, buds, fruit, seeds, and flowers accounting for the remainder of the diet. The analysis of stomach contents by Pham Nhat (1994) found that doucs use at least 50 plant species and have a strong preference for *Ficus* parts, including fruit, leaves, and buds. Group size ranges from 2 to

51 and appears to be dependent on locality, habitat quality, and history of hunting activity in the area (Lippold, 1995, 1998; Nadler et al., 2003; Lippold and Vu Ngoc Thanh, 2008; Rawson, 2009; Fleagle, 2013).

2.4 Black-shanked douc *(Pygathrix nigripes*)

The black-shanked douc (*Pygathrix nigripes*) lives only in Vietnam and Cambodia (Eames and Robson, 1993; Nadler et al., 2003) and is identified by its black legs and arms, grey belly, blue face with yellow eye-rings, and long white tail (Figure 2.1). It is currently listed as endangered "as this species is believed to have undergone a decline of more than 50% in the last three generations (30-36 years, based on a generation length of 10-12 years), due to forest loss and hunting" (Rawson et al., 2008). Nadler et al. (2003) noted that this species is present in southern Vietnam from Kon Tum in the north to Binh Thuan Province in the south (Figure 2.2).

Figure 2.1 Male Black-shanked douc.

Figure 2.2. Range map of Black-shanked douc from the IUCN Red List (Rawson et al., 2008).

Two recent dissertations focus on black-shanked doucs (Hoang Minh Duc, 2007; Rawson, 2009). Dr. Hoang Minh Duc conducted research in Nui Chua National Park, a coastal dry forest, and Phuoc Binh NP, a park with a broad range of habitats including lowland dry dipterocarp forest and wet evergreen forests, while Dr. Ben Rawson studied this species in the Seima Biodiversity Conservation Area, Mondulkiri Province, Cambodia, a mosaic habitat (Rawson, 2009).

Hoang Minh Duc (2007) worked in Nui Chua National Park and Phuoc Binh National Park, Vietnam. These two national parks are located in southeastern Vietnam with Nui Chua located directly on the coast. The research aims for his work were: better understanding of the habitat preference of the black-shanked douc to see how this could affect their distribution, investigating social groupings and group composition, ranging patterns, activity patterns and feeding ecology, looking for other potential habitats within Ninh Thuan Province, and finally the production of maps including the probability of occurrence across the province to potentially expand the protected areas to help conserve these monkeys. Dr. Duc found that the species varied in distribution based on topography, vegetation type, water availability, and elevation. The habitats the doucs inhabited included mountain evergreen forests and very dry open, thorny woodlands; and important features for the doucs were riverine habitats. The doucs showed flexibility in social organization with one-male units forming larger bands during the rainy season. The doucs exhibited this fission – fusion social organization based around resource availability. Activity for the doucs included resting throughout the day with feeding activities in the morning and afternoon, typical of many colobines. The doucs ate young leaves, mature leaves, fruits, and flowers. Nutritional information from the feeding items suggests that doucs were not selecting for protein or trying to avoid foods that were higher in fiber content. The mean crude protein and crude fiber were higher in the non-food leaves then in the leaves selected and ingested. Finally, topographic features were modeled in order to find potential habitat sites for other groups of black-shanked doucs. These models were based on altitude, free water, vegetation suitability, and human habitation and highways. These maps identified forest remnant areas that have potential for douc conservation areas.

Rawson (2009) researched the socio-ecology of black-shanked doucs in the Seima Biodiversity Conservation Area (SBCA), Mondulkiri Province, Cambodia. The aims of his research included: defining activity budgets, social groupings and variation, habitat utilization, and feeding ecology. The habitat preferences of doucs in the SBCA were for evergreen forests and an avoidance of dry deciduous dipterocarp areas. The doucs used mixed deciduous and semi-evergreen habitats during his study, but showed a preference for these more evergreen areas. Rawson reported that the activity budgets of the doucs were very similar to other colobines: they wake early and begin feeding before spending most of the day resting or generally being inactive, followed by a later afternoon period of feeding before moving to sleeping trees before nightfall. The social structures described at the SBCA were based around one-male units, but variation was seen including one-male units, all male groups, lone males, and bands made up of multiple one-male units. He also described instances of larger group formation totaling up to 26 individuals, this behavior has been observed at other sites including Cat Tien NP. He argues that these groups do utilize fission – fusion social organization based on seasonal availability of plant resources. Finally, he reported on the feeding of doucs at SBCA. The most relevant part to this work was the finding that 40% of diet was seed predation. This is the first time that the doucs had been reported to so heavily rely on this resource. The doucs were found to also eat leaves, focusing on young leaves. He goes on to detail the most important tree species in the doucs diet including *Afzelia xylocarpa*, a tree that is a preferred resource for doucs in Cat Tien NP.

2.5 Feeding ecology

Feeding ecology is the study of what an animal eats. More specifically, it examines the suite of characteristics that go into food choice: the chemical, nutritional, morphological, and behavioral aspects of the animal's ecology. More specifically, Milton (2006: 385) defined nutritional ecology, a subset of feeding ecology, as "how an animal deals with the nutritional, spatial, and temporal heterogeneity of the environment to acquire food." Leaf eating monkeys are surrounded by leaves in the forest, but it is essential to be able to separate food items from background scenery (Lucas et al., 2003); therefore, an understanding of food choice can help to elucidate the chemical environment that the doucs live in. To this end, Janzen (1978:73) articulated the desire of field ecologists studying feeding ecology to understand the chemistry of plants in the following way:

> "The plant world is not colored green; it is colored morphine, caffeine, tannin, phenol, terpene, canavanine, latex, phtytohaemagglutinin, oxalic acid, saponin, L-dopa, etc. We now hunger for the details so that we can anticipate their application to our particular case studies. The details are slow to come, because it is hard to know what is in a tree from the viewpoint of a monkey, sloth, or koala, because it is hard to know how much a given amount of herbivory depresses the fitness of a plant, and because it is hard to know what a defense costs a plant."

Leaves are a readily available resource in the environment, but leaves vary dramatically in nutrients, secondary compounds, and other chemical constituents (Janzen, 1978; Milton, 1978; Dominy and Lucas, 2001; Chapman and Chapman, 2002; Lambert, 2011). Researchers now have the ability to quantify the amounts of these compounds and apply them to their particular case studies. However, it is important to note that nutrients, secondary compounds, and animal behavior all influence food selection (Glander, 1982). Freeland and Janzen (1974) described the ability of herbivores to detoxify and remove the effects of secondary compounds by consuming a variety of foods, trying new foods with caution, and by sampling foods continuously. Oates (1977) reported that dietary diversification is an effective strategy for coping with toxins or digestion inhibitors. McNab (1978) discussed the energetics of animals that feed on leaves, a ubiquitous food source.

By understanding the ability of a specific animal to utilize resources that may be toxic to other animals, we can better understand that animal's particular niche. It has been noted that the complex stomachs of some primates allow for the maximization of marginal food resources and also allow some species to process secondary compounds in foods (Glander, 1977, 1978, 1982; Edwards, 1995; Kirkpatrick et al., 2001). Glander (1982) elaborated on the importance of ecological studies focusing on diet composition and food selection in non-human primates, positing that plants are quite capable of defending themselves through chemical means. Glander (1975, 1977) further contended that it was the secondary compounds that were the determining factor in food selection among primates; in fact, secondary compounds can be very important for dietary separation within primate communities (Ganzhorn, 1989). It is important, then, to better understand how primates deal with defense mechanisms that can either be toxic in nature or act to reduce the digestibility of the forage (Van Soest, 1982; Waterman and Kool, 1994; Lambert, 1998).

Diet and feeding adaptations have been central to the study of primate ecology and evolution for decades (e.g. Hylander, 1975; Kay, 1975, 1978; Charles-Dominque, 1977; Terborgh, 1983; Chivers and Langer, 1994). Many of these authors have recognized a series of relationships between anatomy and behavior within primates. It has also been argued that we know less about specific aspects of food choice and the properties of the chosen food than we do about general aspects of anatomy or broad feeding behaviors (Garber, 1987).

This dissertation project is part of a larger body of research on the feeding ecology of primates; therefore it is useful to review the work that has been done. Understanding the difficulties for primates in extracting nutrients from leaves has been a central question in primatology for decades (Glander, 1977, 1982; Janzen, 1978; McNab, 1978; Chivers and Hladik, 1980; Milton, 2006; Kirkpatrick, 2007). Fleagle (1999) briefly reviewed the anatomical specializations of these animals especially their dental morphology and complex sacculated stomach. Kay and Davies (1994) elaborated on the complexities of the colobine digestive physiology, making special reference to dietary variations. While colobines may generally focus on leaves as a main source of food, there is considerable variation within their diets (Kay and Davies, 1994; Kirkpatrick, 1998; Kirkpatrick, 2011). Kirkpatrick (2011) offered the most comprehensive review of this group and found that there is a great deal of variation in their feeding behaviors, especially across habitats; Table 2.1 summarizes some of the information that he provided about the diets of Asian colobines.

Taxa	Habitat	Lf	YL	ML	S/F	Fl	O/U
Pygathrix nemaeus	Tropical Moist Forest	--	--	--	--	--	--
Nasalis larvatus	Peat Swamp	52	42	10	40	3	5
Nasalis larvatus	Mangrove/ Heath Forest	41	38	3	58	--	--
R. avunculus	Tropical Broadleaf Forest	38	--	--	62	--	--
R. brelichi	Temperate Broadleaf Forest	71	--	--	15	7	6
Presbytis hosei	Dipterocarp Forest	78	45	5	19	3	--
S. dussumieri	Moist Deciduous Forest	39	4	35	24	9	3
S. dussumieri	Tropical Broadleaf Forest	58	--	--	29	7	4

Table 2.1: Food Selection in Asian colobines (adapted from Kirkpatrick, 2011). Lf. Leaf; YL, young leaf (a subset of leaf); ML, mature leaf (a subset of leaf); S/F, seed/fruit; Fl, flower; O/U, other or unknown.

Comparing two species of *Rhinopithecus* shows very distinct differences in feeding preferences. *R. avunculus* living in a tropical broadleaf forest feeds predominantly on seeds and fruits (62%) while also consuming a good portion of leaves (38%). *R. brelichi* living in a temperate broadleaf forest feeds mainly on leaves (71%) while supplementing this diet with seeds and fruits (15%). While this contrast is possibly due to the nature of the habitats occupied, it also provides a good example of the dietary breadth within the Asian colobines. In fact, if we look at the diets of a species that lives in a variety of habitats, we also see considerable variation. For example, *Nasalis larvatus* consumes more seeds and fruit and fewer leaves in mangrove / heath forests than in peat swamps. Even greater variation is seen in the diets of *Semnopithecus dussumieri* occupying moist deciduous forest and a tropical broadleaf forest. In the tropical broadleaf forest, leaves dominate the diet while fruits and seeds are used to supplement the diet. Within a moist deciduous forest, the percentage of leaves and fruit and seeds are much closer. It is important to note that, while leaves comprise a bulk of the listed diets, there are also distinctions as to the phase of the leaf consumed, with a focus on young leaves over mature leaves (Milton, 1978; Dominy et al., 2001; Kirkpatrick, 2007). This selectivity for young leaves is based on their physical and chemical properties, which some argue reflects a focus on resources with higher levels of protein to fiber amounts (Milton, 1979; Waterman and Kool, 1994; Yeager et al., 1997; Dominy et al., 2001; Chapman and Chapman, 2002; Zhou et al., 2006; Fashing et al., 2007). Lambert (2011) listed both intrinsic and extrinsic factors that can affect food selection. These factors can be summarized as the physical and chemical nature of the foods or the costs associated with feeding on these items. Janzen (1978) has argued that the conversion of potentially harmful food items to high quality items is important for animals during seasonal variations. Specifically, he addressed the ingestion of high quality food items, which also have high levels of toxins to survive during a stressful period of time (e.g. dry seasons). McKey (1978) explicitly addressed the consumption of nutritionally rewarding items even if they contained high levels of secondary compounds.

2.6 Seasonal resources

Seasonal resource availability has important consequences for primate feeding choice (White, 1998; Wrangham et al., 1998; Lambert, 2011). Kirkpatrick (2011) noted that Asian colobine diets are often quite seasonal. This type of dietary flexibility would be necessary in an environment that experiences drastic changes in forest structure throughout the year because the ability to fall-back onto other resources in the forests allows for exploitation of otherwise non-preferred food resources (Lambert, 2011). As detailed above, the variation in feeding is seen between members of the same species across different habitats, but also within the same species across different seasons. Bleisch and Xie (1998) detailed the seasonal variation in diet for the Guizhou snub-nosed monkey (*Rhinopithecus brelichi*). This species feeds predominately on buds from January to March, switches to leaves from April to June, focuses on fruits and seeds in July through September, and eats a mix of leaf buds, leaves, and flowers during October through December. This illustrates a great diversity of dietary flexibility for an animal commonly characterized as a leaf-eater. It is important to realize the great dietary diversity shown by these animals in seasonal environments.

Because the low available energy content in leaves is hypothesized to pose problems for leaf-eating monkeys, one would predict that folivores would show behaviors associated with energy conservation. Many have noted that adaptations to folivory go well beyond the ability to masticate and swallow leaves (Fleagle, 1999; Milton, 1980). In fact, Montgomery's (1978) edited volume titled, "*The Ecology of Arboreal Folivores*" provided and extensive review of the ecological challenges of folivory. Some of these behavioral adaptations include ranging within small habitat areas and increased resting to digest the low caloric and high fiber diet of leaves. Folivorous primates also need to be able to break down secondary compounds in the leaves that can be toxic (Glander, 1977, 1982). Adaptations to folivory seen in *Pygathrix* are a large sacculated forestomach, and a presaccus, which presumably enhance its ability to digest complex carbohydrates (Edwards, 1995; National Resource Council, 2003).

Chapman et al. (2003) studied the variation of the nutritional values of plants utilized by primates in Kibale National Park, Uganda. Their study raised important issues of variation among and between trees sampled. They also noted that to be as accurate as possible specific trees that animals fed in need to be analyzed rather than any tree of that species (Chapman et al., 2003). These criteria are increasingly used to understand how a species reacts across a varied habitat.

2.7 Resource selection and habitat use

Survival and reproduction are essential with any population, but it is vitally important to understand the resource choices and needs of endangered and critically endangered animals in order to better develop conservation strategies. For example, when Manly et al. (2002) looked at resource selection as a function of resource availability, they noted that it is pivotal to understand the behaviors of an animal to see how it procures resources within its environment and how this aids in its survival and reproduction. Resource selection needs to be defined and quantified within the overall availability of resources. It has been noted that animals will travel to certain areas with higher concentrations of preferred food items to feed (e.g. Milton, 1980), which shows an active selection of a preferred resource, not just a random selection of any food item. This type of distinction has been noted by Manly et al. (2002:1):

> When assessing the feeding ecology of a species, it is necessary to look at not only what and where they eat, but also what they do not eat and where they do not go. The availability of resources is not generally uniform in nature; and use may change as availability changes. Therefore, used resources should be compared to available (or unused) resources in order to reach valid conclusions concerning resource selection. When resources are used disproportionately to their availability, use is said to be selective.

Additional research needs to be done to assess the resource selection and avoidance by the primate taxa that range across Vietnam. As mentioned previously, little is known of the behavior of any of the douc species. As Fleagle (1999:219) stated in his influential text, "*Pygathrix nemaeus*, the douc langurs from Vietnam, Kampuchea, and Laos, are colorful monkeys with little or no sexual dimorphism. Little is known about their natural behavior. They live in mixed, partly deciduous forests and eat buds and leaves." Fleagle (2013) has updated information on the genus, but it is still not an extensive overview of the species. As Covert et al. (2004) noted, the individual identified in Rowe's (1996) picture as a black-shanked douc is actually a grey-shanked douc and this reflects how little was known about these animals. One of the greatest influences in primates' life histories is based upon the food that the primate consumes (Leigh, 1994; Fleagle, 1999). The diet of a primate (or any animal) will influence the growth rate and body size (e.g. Leigh, 1994), and the ranging patterns and general behavior seen in that animal (e.g. Milton, 1981).

2.8 An evaluation of the chemical environment

A number of studies have shown that there is considerable variation in the chemical constituents (nutritional value, secondary compounds) between individual trees of the same species (Glander, 1977; Coley, 1983; Waterman and Kool, 1994; Chapman et al., 2003). As noted above, the protein to fiber ratios of the plant items has been argued to be a very important factor for their selection or rejection as food by animals (Milton, 1978, 1980; Oates et al., 1980; Davies et al., 1988; Chapman and Chapman, 2002; Fashing et al., 2007). Authors have also noted the complications of understanding selection based on secondary compounds (Janzen, 1978; Ganzhorn, 1989; Waterman and Kool, 1994). This continues to be an important area to study as one seeks to understand primate feeding selection and dietary requirements (Freeland and Janzen, 1974; Waterman and Kool, 1994; Chapman and Chapman, 2002; Chapman et al., 2003; O'Brien et al., 2006; Fashing et al., 2007). The adaptation of a species' digestive system to chemical and physical properties of foods is likely to be closely linked to the ecology of the animal (Lambert, 1998). In addition, behavioral data on food selection is used to understand seasonal resource use and the amounts of seeds and fruits within an animal's diet (Waterman and Kool, 1994; Kirkpatrick, 2011). Plants have been sampled and analyzed for the following chemical components hypothesized to have the greatest impact on primate nutrition: crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF), ash, total phenolics (TP), condensed tannins (CT), calcium (Ca), Potassium (K), Magnesium (Mg), Sodium (Na), Phosphorus (P), Copper (Cu), Iron (Fe), Zinc (Zn), and Manganese (Mn). Soil samples have been analyzed for the following components: nitrate nitrogen (NO_3-N), Potassium (K), Phosphorus (P), Magnesium (Mg), Aluminum (Al), Sulfates (SO_4), Ammonia Nitrogen (NH_4NO_3), Nitrate (NO_2), Nitrogen (N), Chlorides, Ferric Iron (Fe), Calcium (Ca), Humus, and pH.

2.8.1 Plant chemistry

I now detail the chemical constituents of plants used in understanding colobine dietary ecology. Waterman and Kool (1994) stated that plants are not simply discrete packets of nutrients for primates; as they remark, plants "also contain a range of metabolites that are, variously, refractory to digestive processes, capable of lowering efficiency with which nutrients can be obtained, or actually harmful to the animal (1994:251)." Therefore, an understanding of the plant chemistry is imperative for understanding food selection in these specialized primates. Lambert (1998, 2011) outlined the challenges faced by primates to select foods while avoiding high levels of secondary compounds. Interestingly, toxicity can be variable within food items. Janzen (1978) defined toxicity as a measure of the nutritional benefit of eating an item, even if there are high levels of secondary compounds (e.g. eating a seed with a higher protein content than a leaf with a lower protein content if both have the same amount of secondary compounds).

2.8.1.1 Protein

Protein is required by all animals to aid in growth and the maintenance of body tissues. Crude protein (CP) is measured from the amount of nitrogen present in the sample multiplied by a conversion factor of CP6.25 (NRC, 2003) using the macro-Kjedahl method (Williams, 1984). This has been the standard conversion factor for feeding studies, although Conklin-Brittain et al. (1999) have also suggested a lower conversion factor to more accurately describe protein contents of foods by taking in account the actual availability of the nitrogen. The authors noted that the conversion of CP6.25 rather than CP4.3 does not significantly vary for fruits and flowers, but may be important for leaves. The difference between the two conversion methods limits the measure of lignin bound nitrogen within the sample. As this is a conversion factor done after the chemical sampling, both conversions can be formulated and analyzed based on the ecology of the animal. This calculation can have the greatest effect on trying to properly balance the nutrient requirements for captive animals; however, Conklin-Brittain et al. (1999) do not completely discount the CP6.25 conversion concluding that it is an adequate measure of protein content.

2.8.1.2 Fiber

Fiber represents the structural portions of cell walls in plants and is the digestion resistant portion of the plant (Lucas et al., 2003). While this portion of a plant is not accessible to all herbivores, the complex stomach of colobines allows them to exploit some of the fiber available in plants. Milton (1980) noted that a critical difference between species of primates is the efficiencies at breaking down the structural carbohydrates in plant materials. Fiber can be described by constitute indigestible parts within the plant: cellulose, hemicelluloses, and lignin. Hemicelluloses can yield energy, but only after gastrointestinal fermentation; in contrast, lignin cannot be digested (NRC, 2003). Fiber is measured by two methods: neutral detergent fiber (NDF) and acid detergent fiber (ADF) (Van Soest et al., 1991). Neutral detergent fiber measures cellulose and lignin and most hemicelluloses while acid detergent fiber measures cellulose and lignin (Lucas et al., 2003). Wrangham et al. (1998) noted the use of both of these tests in order to understand the complete fiber content of the cell walls of plants. Lower fiber and higher protein levels have been cited as a major selective factor for primates' foods (e.g. Milton, 1980; Oates et al., 1980; Waterman and Kool, 1994; Chapman and Chapman, 2002).

2.8.1.3 Phenolics

Phenolics are compounds that contain six-membered carbon rings with hydroxyl (OH-) groups (Lucas et al., 2003). Waterman and Mole (1994) noted that phenolics aid in defense functions of plants against herbivory. The levels of phenolics are measured using the Prussian blue test (Hagerman, 1998). The results are measured against gallic acid, which acts as the standard. Gallic acid provides a standard six-point curve for analysis. The amount of gallic acid in the sample is measured with a spectrometer at 700nm (Lucas et al., 2003). This method converts the sample to a microassay. Lucas et al. (2003) noted that this test is not an absolute measure of the phenolic concentration, but the reducing power of the phenolics.

2.8.1.4 Tannins

Tannins are a type of phenolic compound found throughout the plant kingdom (Dominy et al., 2001). Tannins can interfere with protein uptake in animals by binding to the protein, making it inaccessible during digestion (Lambert, 1998). The range of reactions to tannins includes reducing digestibility of materials, toxic poisoning, and death (Milton, 1998; Dominy et al., 2001). Janzen (1978) also described the two types of tannins: nonhydrolyzable tannins used mostly for plant defense, and hydrolyzable tannins used to select against certain organisms. Tannins are also a fungal inhibitor and have been hypothesized by Janzen (1978) to help increase the uptake of fruit by mammals to disperse seeds. Oates et al. (1977) found that *Colobus guereza* favored plants that were lower in tannin content. Wrangham et al. (1998) investigated the antifeedant chemical properties of foods selected by chimpanzees and cercopithecines in Kibale National Park, Uganda. Antifeedants are specific chemical or physical properties of plants that allow them to protect themselves from herbivorous animals. The authors noted that physiological adaptations to antifeedants were likely to be very important due to the nature of tannins to binding proteins, thus making them unavailable to the animals.

2.8.1.5 Dry matter and ash

Dry matter is the percent of the sample that is not water. This can be a very useful measure for comparison of samples. The ash content is the overall inorganic portion of the sample (Ortmann et al., 2006). Placing the sample in an oven and removing all of the water content will give the weight difference and amount of ash present in the sample.

2.8.1.6 Mineral content

Minerals are the inorganic elements that perform essential functions in living organisms (Lambert, 2007). The mineral content of potential foods has been shown to influence selection or rejection of certain items by primates (Hladik, 1978; Nagy and Milton, 1979; Yeager et al., 1997; Fashing et al., 2007). Fashing et al. (2007) noted that, within black-and-white colobus, there does appear to be some selection of food items based on their mineral content for example; ingestion of zinc seems to be driving selection of forage.

2.8.2 Macrominerals

2.8.2.1 Calcium (Ca)

Calcium is used in the construction of skeletal material and teeth. This element is required in large concentrations to help maintain healthy body systems, and more specifically calcium binds and activates cellular proteins (NRC, 2003). Calcium is also very important for lactating females (Lambert, 2007).

2.8.2.2 Potassium (K)

Potassium is used for the maintenance of the acid-base balance and osmotic pressure (NRC, 2003). Yeager et al. (1997) reported that young leaves of preferred food sources ingested by *Nasalis larvatus* contained significantly elevated levels of potassium compared to mature leaves of non-preferred food sources.

2.8.2.3 Sodium (Na)

Sodium is used to regulate total body water and thirst in mammals (NRC, 2003). Oates (1978) noted that sodium levels are typically lower in the diet of herbivorous animals, which may cause an organism to select foods or minerals with sodium in soils to maintain the necessary levels of this element.

2.8.2.4 Phosphorus (P)

Phosphorus is required by the body to help with metabolic and energetic reactions within cells (NRC, 2003). Yeager et al. (1997) additionally reported that the young leaves selected by *Nasalis larvatus* contained elevated levels of phosphorus.

2.8.3 Trace elements

2.8.3.1Copper (Cu)

Copper is important for a number of enzymes and proteins in mammalian cells (NRC, 2003). Linder (1996) noted that copper is important for the functioning of melanin formation and the formation of connective tissues.

2.8.3.2 Iron (Fe)

The body uses iron in the production of proteins, specifically hemoglobin, myoglobin, and ferritin (NRC, 2003). In addition to the important function of oxygen transport in the blood stream, iron also assistances in the metabolism of several nutrients (NRC, 2003).

2.8.3.3 Zinc (Zn)

Zinc is linked to proper metabolic function with health implications for growth, cognition, and reproduction (King and Keen, 1999). Fashing et al. (2007) reported that eastern black-and-white colobus (*Colobus guereza*) in Kakamega Forest, Kenya selected leaves, in part, based on zinc content.

2.8.3.4 Manganese (Mn)

The body uses manganese to regulate enzymes and enzyme systems. This regulatory function extends to muscles, nerves, and metabolism of other macronutrients (NRC, 2003).

2.9 Soil Chemistry

The chemical nature of soil has direct implications for plant communities (Janzen, 1974; Oates et al., 1990). The analysis of the soil chemistry informs us about the nutrients available to the plants and how they invest these chemicals. Coley and Barone (1996) noted that leaves in tropical forests are lower in nutritional content and higher in secondary compounds than in temperate forests. Plant chemical composition can also be affected by the resources available to the plant through the soil it is growing in. Janzen (1974) explored the idea that related properties of habitats are built upon the soils that these communities are growing in. Forest productivity should be able to be quantified based on the nutrients available in the soil. Gartlan et al. (1978) noted that the features of soil chemistry affect the plant community, which then has direct consequences for the primate community. Additionally, the measure of secondary compounds may be influenced be the soil the plant is growing in (Gartlan et al., 1978; Oates et al., 1990). Janzen (1974) studied the soil composition and associated plant and animal communities in blackwater habitats. This study not only looked at the nutrient composition of soils and plants but also was focused on finding limiting factors such as nutrient availability or higher levels of secondary defense compounds. Workman (2010) worked on limestone islands in northern Vietnam and tested food items eaten by Delacour's langur (*Trachypithecus delacouri*). As with

Janzen, this study looked at nutrient availability in soils to see how this may translate to nutrient availability for feeding items. Workman reported that the most important items in the diets of the langurs were not endemic karst plants, that while the soil had a high percentage of carbon there were young (preferred) leaves readily available, and plant defenses and fiber levels did not seem to influence leaf selection.

Wilde (1958) researched plant and soil interactions in controlled nursery settings. Even within this controlled setting, there are numerous applications to natural forests. Wilde described how nutrients contribute materials to produce cell walls and protoplasm necessary to plant growth, as well as describing the importance of counteracting toxic materials and providing for the basic metabolic functions of the plant. He also noted that early research in soil chemistry lead to inflated results for chemical analysis of soils, but newer techniques allowed for more accurate measurements. These measurements allowed researchers to elucidate relationships between soil chemistry and plant growth. The importance of soil chemistry rather than soil physical properties became important after researchers realized the chemicals, not the physical properties of the soils, effected plant growth. Wilde noted that ten elements and five minor (trace) elements are necessary for plant growth. The elements are: carbon, oxygen, hydrogen, nitrogen, sulfur, phosphorus, potassium, calcium, magnesium, and iron. The trace elements are: boron, manganese, zinc, copper, and molybdenum. Trace elements are important to plant metabolic function as they act as catalysts for both chlorophyll and carbohydrate synthesis. While trace elements are very important for the growth and development of plants, higher concentrations of these elements can be harmful or extremely toxic.

Due to the seasonality of the forests in Cat Tien National Park, we can measure variations within the soil nutrients from the dry to the rainy seasons. Wilde (1958) noted that the variability of the moisture content of soils can interfere with plant uptake of nutrients due to the behavior of exchangeable ions. The exchangeable ions are how the root systems of the plants are up taking these vital nutrients.

Davies and Baillie (1988) studied the consumption of soil by *Presbytis rebicunda* in Borneo and reported that the animals did not go down to the ground to eat the soil, but instead took it from termite-mounds that were located near the base of the trees. Importantly, the authors noted that the consumption of the soil could serve a number of functions, from treating digestive ailments to supplementing nutritional requirements (Davies and Baillie, 1988). Fashing et al. (2007) discussed geophagy (eating soil) in relation to African colobines. They suggested that this behavior appeared to mitigate secondary compounds as well as supplement nutrients in their diets (Oates, 1978; Davies and Baillie, 1998). This could have interesting implications for animals living in dry or very seasonal environments.

2.9.1 Elemental Analysis:

The three elements analyzed in my research were nitrogen (N), phosphorous (P), and Potassium (K). Each of these elements is important in plant growth and development. Hesse (1971) noted that plant growth is limited most by available nitrogen. Nitrogen is very important for the proper growth and development of plants because it provides the essential constituents of proteins. Some of the characteristics thought to be related to nitrogen deficiencies in plants are stunted growth, premature death of leaves and buds, and an underdeveloped root system (Wilde, 1958).

Phosphorus is important in the growth and development of plants as a catalyst for respiration reactions, the maintenance of meristematic tissues, and the transformation of carbohydrates into useable material (Wilde, 1958). Meristematic tissue is cells or groups of cells that have the ability to divide; these tissues are found near the tips of roots and stems or in the buds of plants (Harris and Harris, 2001). Wilde (1958) noted that a deficiency of phosphorus can cause plants to have underdeveloped root structures and higher seedling mortality.

Potassium is used by plants during photosynthesis to help speed up the use of carbon dioxide and helps with other important metabolic functions (Wilde, 1958). Potassium deficiencies are noted to cause lack of root development and normal foliage.

2.10 Parasite ecology

Huffman (2007) noted that a primate's life is closely linked with the parasites that it carries. Moreover, an animal may be infested with parasites for a number of reasons, but this often does not provide any health risk to the host (Ancrenaz et al., 2003). Altizer et al. (2003: 520) defined parasites as, "any infectious organism capable of colonizing a host, utilizing host resources, and spreading to new hosts." Primates come into contact with parasites through water resources, sleeping sites, and vegetation used for traveling or feeding (Freeland, 1980; Huffman, 2007; Kenyon, 2007). Huffman and Chapman (2009) stated humans share a number of parasites with monkeys and apes. This potential for interspecies exchange can have very important consequences as these groups continue to utilize the same landscapes and have increased interactions amongst them. Some of these consequences are the interchange of viruses, which can trace their origins to zoonotic transfer from apes to humans (Ebola virus) or other infectious diseases such as the HIV virus. With a loss of biodiversity, Keesing et al. (2010) argued that there is the possibility of disease transmission increasing as well. This is a factor that may well come into play within the decreasing forests of Vietnam as the human population continues to expand and have more contact with nonhuman primates.

Understanding what parasites a given taxon hosts allows for an investigation of their relationships. Lorzano (1991) noted that parasite infection could alter foraging behaviors of animals and listed three influences to diet choice: avoidance of certain food items, ingesting certain foods to change gastrointestinal composition, and self-medication to kill established parasites. Field scientists have observed primates self-medicating with plants and engaging in geophagy to help reduce parasite loads (Glander, 1998; Huffman, 2007). Wrangham and Nishida (1983) discussed the ingestion of selected plants by ill chimpanzees. Janzen (1978) suggested secondary compounds found in plants ingested could be beneficial to the removal of intestinal parasites. Hoang Minh Duc et al. (2011) noted that some of the foods selected by black-shanked doucs are used in traditional Vietnamese medicine. If these foods were being selected to help mitigate parasite loads, then it would be interesting and informative to know which parasites are infecting them and if this is a site specific or similar across their entire range from eastern Cambodia to the coast of Vietnam. Doucs have also been recorded ingesting soil at a salt lick in Cambodia (Rawson and Luu Tuong Bach, 2011). Rawson and Luu Tuong Bach (2011) suggested that this type of geophagy may be used for toxin reduction or to add essential minerals to the diet; however it could also be used to help reduce parasite loads.

2.10.1 Parasite and host interactions

While this dissertation does not focus on the life cycles of parasites, a brief introduction to some of this material is of interest. Nunn and Altizer (2006) stated that parasites cost the host not only material resources, but also will exploit behavior and metabolic function. Combes (2011) contrasted predator prey relationships with parasitic relationships as a difference of short term and the death of the animal (predator- prey) compared to a “durable interaction” that usually does not result in the death of the host (parasitic). Parasites may go through many different hosts before completing their life cycle and reaching reproductive age (Roberts and Janovy, 2008). Some of the types of hosts available to parasites are definitive hosts, intermediate hosts, paratenic or transport hosts, and reservoir hosts (Roberts and Janovy, 2008). A definitive host is where the parasite reaches sexual maturity. An intermediate host is where a stage a parasite must pass through before becoming sexual mature, but is not the definitive host. A pararenic host is characterized by the parasite that does not undergo any maturation, but stays alive and is still infective. Finally a reservoir host is an animal that can have an infection and pass it on to humans. The concept of a reservoir host is interesting when there are many sympatric species living in fragmented habitats. Many of these definitions have been created with humans as the infected host, but we can still use these definitions for other non-human primates. Chapman et al. (2005) noted that with increased forest fragmentation non-human primates had elevated levels of gastrointestinal parasites than in less anthropogenically altered landscapes. Gillespie et al. (2005) also found that red-tailed guenons had greater levels of multiple infections living in logged versus undisturbed habitats. These greater areas of contact between species lends toward higher infection rates.

2.10.2 Seasonality

The parasite samples analyzed in this dissertation were collected in May, a transitional month in Cat Tien NP coming at the end of the dry season and beginning of the wet season. It is possible for the rains have started at this time, but the seasonal flooding of the low lying and riparian areas has yet to occur. While seasonal affects of parasite loads cannot be contemplated in this research due to parasite samples only being available from a single month, many studies have focused on variation of parasitic infection during seasonal changes (Huffman et al., 1997; Kalema Zikusoka and Rothman, 2005; Kenyon, 2007; Chapman et al., 2009; Loudon, 2009). In addition, animals that have more frequent contact with humans or domesticated species run the risk of higher rates of parasite infection (Ekanayake et al., 2006). Seasonal changes in the forest resource availability effect ranging patterns of animals, causing greater contact between species as they move about for food or shelter.

Huffman et al. (1997) discussed the importance of understanding the life cycle of the parasites in order to better understand the likelihood of infection. The study of seasonality effects on infection rate were shown to have a significant difference between the wet and dry seasons with chimpanzees having greater infection rates of *Oesophagostomum stephanostomum* in the wet season (Huffman et al., 1997). This study also raised an interesting perspective on concurrent infections as a factor in animal deaths due to immunosuppression. The parasitic infections were severe enough that the animals contracted other diseases. Nutritional stress has been shown to be an exacerbating factor in parasite infections (Poppi et al., 1990; Gulland, 1992; Sumbria and Sanyal, 2009). Hanssen et al. (2004) studied the costs and effect of immunosuppression on a host for disease response in times of stress or malnourishment. Chapman et al. (2005) noted the synergy between nutritional status and parasite loads within primates. An animal in nutritional stress can be more at risk to parasitic infection. Host ruminants have been shown to be more resilient to gastrointestinal parasite infection when higher levels of protein are present within the diet (Van Houtert and Sykes, 1996).

2.10.5 Previous Parasite Studies from Cat Tien NP

Kenyon (2007) conducted an analysis of parasite loads focusing on yellow-cheeked crested gibbons (*Nomascus gabriellae*) in Cat Tien NP. She also included samples from black-shanked doucs (n = 8), stump-tailed macaques (*Macaca arctoides*) (n = 3), long-tailed macaques (*Macaca fascicularis*) (n = 2), pig-tailed macaques (*Macaca nemestrina*) (n=4), pygmy lorises

(*Nycticebus pygmaeus*) (n = 1), wild pigs (*Sus scrofa*) (n = 1), and mouse deer (*Tragulus javanicus*) (n = 1) in her analysis. This study concluded that gibbons had many parasitic infections from nematodes, protozoa, and other unidentified parasites while fewer nematodes and protozoa parasitized black-shanked douc. Kenyon's (2007) study provides data that can be compared with this current research.

2.11 Conservation in Vietnam

Vietnam is a densely populated country home to over 92 million people with a total land area of 329,560 km^2, or about the size of New Mexico (CIA factbook, 2013). Vietnam is part of the Indo-Burma hotspot and is home to an impressive diversity of animals and plants with 109 large mammal species, 850 species of birds, and 9,600 to 12,000 species of plants (Sterling et al., 2006). Additionally, Vietnam is of great importance to primatologists due to the high numbers of primates (Nadler et al., 2003).

Mittermeier et al. (2013) reported that 26 primate species are found in Vietnam, including two lorises, six macaques, twelve colobines, and six gibbons. The conservation status of these species is: one species "not evaluated," two species are "least concern," one species is "near threatened," seven species are "vulnerable," eight species are "endangered," and six species listed as "critically endangered." (Table 2.2) Mittermeier et al. (2012) lists five taxa from Vietnam among the world's 25 most endangered primates: *Trachypithecus delacouri, T. poliocephalus, Pygathrix cinerea, Rhinopithecus avunculus,* and *Nomascus nasutus*.

Conservation work and research needs to not only focus on the individual parks and species, but can benefit from a broader perspective that incorporates humans into the landscape. Wu and Hobbs (2002:361-362) clearly highlighted the benefit of a landscape ecological perspective,

> "One of landscape ecology's greatest contributions to our understanding of landscape pattern and process is the inclusion of humans into the landscape. Landscape ecology focuses on relatively large-scale ecological systems that are increasingly influenced and determined by human activities. As most unequivocally indicated, socioeconomic processes are the primary drivers for land use and land cover change, which in turn determines the structure, function, and dynamics of most landscapes. Therefore, it is evident that humans themselves and their activities (be they rational or radical) must constitute an integral part of the ecology of landscapes, and they should be treated as such in research. In addition, the ideas relating to landscape planning and design need more careful interweaving with the biophysical aspects of landscape ecology, particularly if we aim to allow landscape ecology to be forward looking and to assist in designing landscapes for the future to prevent the recurrence of current land use dilemmas".

Conservation needs to be placed within a social context by considering the interests of local people, park administrators, animals, NGO's, and tourists. This project focuses on one park in particular; however, many of the concerns of this park can be extrapolated to the country as a whole, in a sense offering a microcosm of the successes and failures that face conservation within Vietnam. Conservation goals must address the humans that will be affected by these laws.

Vietnam has made a strong commitment to conservation, a commitment that it is trying to honor in the face of a number of social and political issues. In fact, the Cat Tien National Park Conservation program had a very strong community outreach and education portion. This program, as well as other education programs, is mentioned for the benefits they provide to the park, tourists, and the local community. There are also large numbers of people that live in areas bordering national parks. The International Centre for Environmental Management (2003) reported that between 1,457,536 and 2,579,168 people lived around the border of Cat Tien NP. Nguyen et al. (2005) noted that most of the people living inside and around Cat Tien relied on agriculture to survive and also exploited forest resources when available; this exploitation of forest resources is an issue of great importance for the future of Vietnam's natural treasures. Non-timber forest products and animals are also routinely exploited from the forests of Vietnam, but the demographic of use is changing in part due to the rising per capita income in Vietnam, which is reported as $1550 (World Bank, 2013). This rise in the per capita income can be used to illustrate the vital concern that exploitation of forest products it is no longer only based solely on subsistence: more disposable income means a greater demand for these illegal products (Milliken and Shaw, 2012).

2.12 Cat Tien National Park

2.12.1 Park Description

Cat Tien National Park is 150 km north of Ho Chi Minh City. It was designated a protected area in 1978 (originally as Nam Bai Cat Tien) and initially was 38,000 ha in size and located in Dong Nai Province (Pham Nhat et al., 2001). In 1998, the park was expanded to 73,878 ha by areas added from Lam Dong and Binh Phuoc Provinces (Polet et al., 2004). Pham Nhat et al. (2001) stated that Cat Tien contains one of the last evergreen and semi-evergreen lowland rainforests in Vietnam. Murphy and Phan Duy Thuc (2002) also noted that Cat Tien is a very important habitat for black-shanked doucs. The park is located at the junction between two major ecosystems in Vietnam: the southern end of the Dalat Plateau and the Mekong Delta (Polet and Ling, 2002).

Monastyrskii (2000) described the climate in Cat Tien as being a seasonal system with annual monsoons. There are two distinct seasons: a wet season that ranges from May through November and a dry season from December through April. The annual rainfall for Cat Tien is 2.5 meters (Nguyen et al., 2005), with most of this coming during the rainy season. In addition to the faunal diversity of Cat Tien, the floral communities of this park are quite diverse. There are seven distinct forest types located within the park: evergreen forest, semi-evergreen forest, bamboo forest, mixed tree and bamboo forest, brush and scrub, grassland, and wetlands and lakes (Monastyrskii, 2000). This diversity of habitats as well as its biogeographical location lends itself well to significant endemism and diversity (Sterling et al., 2006). In an effort to better understand the restoration of forest and land rehabilitation, Blanc et al. (2000) surveyed Cat Tien for the structure and floristic composition focusing on the successional trends within the park. The study found that *Lagerstroemia* and *Dipterocarpus* dominate the forests of Cat Tien and that the succession of the forests is based on the soil compositional structure and water content (Blanc et al., 2000). The soil composition is important for the floristic structure and it would be very interesting to see how it is influencing the nutritional content of the leaves within the forest. The dynamic structure of the forest is very important to understand given the history of defoliates and logging within the forests of Vietnam. A final cautionary note by Blanc et al. (2000) dealt with the possibility of exotic species that could change the forest structure and composition. The additional planting of exotic species along the borders of the national park could be influencing the edge communities and negatively impacting the forest structure.

Pham Nhat et al. (2001), a field guide of mammal species present in Cat Tien, reports that the park is home to a diverse community of primates including the pygmy loris (*Nyciticebus pygmaeus*); four species of macaques: the bear macaque (*Macaca arctoides*), long-tailed macaque (*M. fascicularis*), pig-tailed macaque (*M. leonina*), and the rhesus macaque (*M. mulatta*); the silvered langur (*Trachypithecus cristatus*) [now recognized as *T. margarita*]; the black-shanked douc (*Pygathrix nigripes*) and one ape species, the yellow-cheeked crested gibbon (*Nomascus gabriellae*). Cat Tien is home to the Asian elephant (*Elephas maximus*), numerous ungulates, and a large number of migratory bird species. Clearly, then, Cat Tien is very important to conservation for a number of reasons. The park has an established record of conservation actions and has received substantial international funding to conserve its natural resources. In 1998, The Cat Tien National Park Conservation Program was established with joint funding of $8 million USD from the World Wildlife Fund for Nature (WWF) and the Government of Vietnam. The second major funded project was the $32 million USD World Bank Forest Protection and Rural Development Project that operated in the buffer zones of Cat Tien and Chu Mom Ray NP. Cat Tien is one of the best-protected national parks in Vietnam with a well-trained forest ranger and scientific section. Finally, in contrast to many protected areas in Vietnam, Cat Tien is a national park where you can see and hear animals on a regular basis. This speaks volumes to the levels of protection afforded the wildlife within the park's borders.

2.12.2 Goals of Cat Tien National Park:

The goals of Cat Tien are clearly laid out by Nguyen et al. (2005: 5) and worth listing exactly as envisioned:

1) Maintain forest and wetland ecosystems.
2) Preserve rare fauna and flora gene resources, such as the Java rhinoceros, elephant, and other rare animal species.
3) Protect natural landscapes, implement scientific studies, and encourage conservation education.
4) Develop eco-tourism, stabilize surrounding settlements to create more jobs and improve standard for local residents.
5) Protect the Tri An hydroelectric plant.
6) Develop scientific studies in order to contribute to conservation.

The following paragraphs elaborate on the stated goals of Cat Tien National Park. National parks in Vietnam are protectionist by nature and it is illegal to exploit forest resources within the boundaries of a national park without exception. This can cause strife at the borders of national parks between people living at the forest's edge and the national park staff. It is the goal of the national park to protect the forest ecosystems, but accommodations are often made to help ease relationships with local peoples surrounding the park.

A second stated goal of the national park is the maintenance of forest and wetland ecosystems. The maintenance of these areas is of paramount importance for any conservation planning. This goal also establishes a base line for any development work within the area, it sets clear boundaries and distinctions to areas that cannot be disturbed.

The maintenance of the wetlands is not only important for the bird resources that occupy the area, but also to provide a source of water for agriculture and people downstream. The Dong Nai River forms the northern and eastern boundary to Cat Tien National Park. The river serves as an important boundary but also is used extensively by the surrounding communities for agriculture and transportation. The depth of the river fluctuates between 2-3m in the dry season to a high of 8m during the wet season (Nguyen et al., 2005). The forest areas surrounding the river are seasonally flooded and some of the wells used by local communities dry up during the dry season (personal communication Mr. Ha Khanh Chau). It has been hypothesized that the distribution of water resources can be one of the limiting factors for animals on this landscape. The seasonal flooding can also have negative effects on the local human populations by flooding homes and agricultural fields. Additionally, the reservoir of Tri An and its hydroelectric power station give power to the region. The Tri An hydroelectric plant is downstream from Cat Tien National Park and protection of the wetlands and rivers in Cat Tien directly benefit the power plant. This is of vital importance to Vietnam's rapidly growing economy through watershed management and the creation of dams to supply power (Martin, 2004).

The fauna of Vietnam are protected in accordance with the signing of multiple international accords and the establishment of various laws and regulations. These documents protect the animals and the natural landscapes where they live. While the development of eco-tourism in these areas can be problematic, I believe that, if the park administration really wants to follow the other goals based on the protection of the environment, then maybe the tourism in the area could be ecologically sound, not simply tourism that takes place near nature.

The desire to incorporate scientific studies to further conservation planning and work to meet the goals for the park is very important. Cat Tien has worked with international conservation organizations focusing on crocodiles, rhinos, golden-cheeked crested gibbons, and wild bovids. These previous research relationships, coupled with previous field work on black-shanked doucs (O'Brien et al., 2008), set the stage for the long-term field research on the ecology and conservation of the primate community in Cat Tien National Park that is reported in this dissertation.

One subject that has not been discussed in sufficient detail in relationship to conservation at Cat Tien is the humans on that utilize this landscape. It can be very easy for field scientists to lose themselves within their study species, but this can be shortsighted if we really want to have sustainable conservation strategies. There are 11 ethnic minorities located within the area and each group has its own perceptions of the forest and resource use. This dissertation does not discuss these groups, but it is key to acknowledge that the humans on the landscape are an important consideration to any conservation or development plan. This fits in quite well with the park's fourth stated goal of trying to improve the livelihoods of local peoples that live near the parks borders.

Cat Tien National Park was made part of the United Nations Educational, Scientific, and Cultural Organization (UNESCO): Man and Biosphere Programme in 2001. This program is devoted to reducing biodiversity loss while improving the livelihoods of people in the area. The program seeks to enhance the social, economic, and cultural conditions for environmental sustainability. It also listed a number of threats to biodiversity within Cat Tien including the conversion of forestland into agricultural land, fragmentation of forest sectors into smaller areas, and poaching of animals within protected areas (UNESCO, 2001). This series of anthropogenic changes brought on by agriculture and forest exploitation are serious concerns for the future of conservation within this area.

2.12.3 Educational campaigns

Cat Tien National Park Conservation Program developed a number of educational campaigns and used a range of approaches to deliver this information to the local communities. A number of small books were published including: “Learn about the forest” and “What are all of those trees doing in the forest.” These books are published in both Vietnamese and English and are meant to educate children about the forest and the animals that live within the forest. Their main goal seems to be to instill a sense of ownership and stewardship over the forest. Both of these books end with simple messages of how to save the forest and protect it for generations to come. I feel that this is a very valuable message and means to spread this message. There are also nature walks for local school children, and the headquarters of the national park boasts very nice interactive displays encouraging visitors to become more informed about the park to better enjoy their visit.

Another method that is used is the inclusion of educational materials in scientific surveys. Nguyen et al. (2005) discuss the two main objectives of a joint US Fish and Wildlife Service and

Cat Tien National Park program for the status of golden-cheeked gibbons. The first goal was to better understand the conservation implications within the protected area. The conclusions they arrived at were similar to other surveys that have been carried out throughout Vietnam: the main threats to the species were habitat loss, illegal hunting, illegal logging and non-timber product extraction, and agricultural activities.

The second goal was a strong educational outreach. This outreach included: printed materials to hand out to local school children, a conservation competition, and community education that included 36 communes and villages. This was a method to involve the local community and raise awareness surrounding the park. I think that this is a very important portion of conservation awareness and naturally includes the local community.

Borgerhoff-Mulder and Coppolillo (2005) argued that conservation is all about choices and this is certainly relevant to this study. The fauna and flora of Vietnam can be conserved. It will take a concerted effort on the parts of many people: the central government of Vietnam, the provincial governments at the local level, the local communities, park staff, NGO's, and scientists both in country and international. Vietnam's protected areas are protectionist by design, but there may be some flexibility in practice within individual parks. This ambiguity may be what is needed to have local successes, but can be a slippery slope to follow in practice. In order for conservation to succeed, I feel there needs to be a strong community education component and buy-in from the local community. The greater development of ecotourism activities could greatly benefit both the national park and the surrounding communities. There will need to be great attention to how this is accomplished, so that there is actually ecological tourism and not a form of tourism that will be detrimental to the environment.

Borgerhoff-Mulder and Coppolillo (2005) also noted the importance of understanding the science behind conservation activities. Cat Tien National Park faces many of the same conservation issues faced by many protected areas around the world including human encroachment, funding shortages, illegal hunting, and the gathering of natural resources. I believe that the park is in a good situation with government and local support to make a difference in the lives of the local communities and the animals that reside there. The park had hosted a conservation workshop from April 27- 29, 2007 on the status of rhinos (Nguyen Thuat, personal communication) and garnered funding for a primate conservation center to be located onsite. I will use these two examples to illustrate current conservation actions in Cat Tien NP.

These were events that have led to both a positive and a negative result for conservation in Cat Tien NP. On a positive note, the Dao Tien Primate Species Center is celebrating its seventh year of operations and has seen the successful rehabilitation and release of yellow-cheeked gibbons, black-shanked doucs, and pygmy lorises. Dao Tien is supported by EAST (Endangered Asian Species Trust) and is actively involved in the animal rescue and rehabilitation trade, and also in the education of national and foreigners alike. Dao Tien continues to work with the local and national government of Vietnam to protect and conserve wild species in the region.

On the other hand, the case of the Javan rhinoceros shows a failure in conservation. Brook et al. (2011) report on the extinction of the Javan rhinoceros in Vietnam. Cat Tien NP was home to the last individuals of this subspecies of rhino on mainland Southeast Asia. The final individual was poached in 2009 and, when part of the skeleton was recovered in April 2010, it was noted that the horn had been removed and there was a bullet in its forelimb (Streicher et al., 2010). Brook et al. (2010) stated that poor protection and law enforcement contributed significantly to the extinction of this species. WWF and Cat Tien NP had been working on the conservation of this species since it was rediscovered in 1988 as a hunter was trying to sell the horn and skin of a poached individual (Schaller et al., 1990). The last two decades have seen numerous surveys and projects to help conserve this species. The final survey by WWF was conducted from October 27th 2009 until April 8th 2010 in an attempt to collect fecal samples using trained dogs to determine how many individuals still lived in Cat Tien NP (Brook et al., 2011). While this survey successfully collected fecal samples at the beginning, ultimately the survey would result in finding the final individual dead. The fecal samples collected during this research were genetically matched to the remains collected from the final individual (Brook et al., 2011). The witnessing of an extinction event is clearly a somber moment in conservation efforts, but we cannot give up and allow these challenging times to try our resolve. We can help to affect change and conserve species and habitats around the world.

Cowlishaw and Dunbar (2000: 398) noted the importance of unique solutions for conservation issues. "Unfortunately, conservation problems are too complex and too variable to yield to one simple universal solution. In fact, most conservation problems are likely to benefit from a mixture of tactics that are carefully chosen and integrated based on the precise nature of the problem being dealt with. Ultimately, each conservation problem will have its own unique solutions."

2.12.4 Conservation efforts

The signing of multiple international accords protects and preserves the fauna and flora of Vietnam. Regulations governing national parks in Vietnam are designed to provide absolute protection of their biodiversity; thus, it is illegal to exploit forest resources within their boundaries without exception. Disallowing the local peoples' previous legal exploitation of such resources can cause strife at the borders of many parks in Vietnam. Many of Vietnam's forest habitats are fragments that are often quite small, and are under heavy utilization by people. Chapman et al. (2007) discussed the conservation value of fragmented areas. The authors found that when the fragments are under constant pressure from local communities, the primate populations face extirpation. However, the authors are hopeful that successful conservation programs and strategies that help the local peoples can save these fragments and their primates.

2.13 Contributions to Anthropology:

This project contributes to anthropological theory and to the multifaceted nature of the discipline of anthropology. Specifically, this work can be considered to be at the interface of conservation biology and anthropology as described by Mulder and Coppolillo (2005). They reviewed many conservation projects that are a successful joining of biological and cultural anthropology. The importance of working with people directly affected by conservation efforts is essential for the long-termed success of the research proposed here. While the focus of this research is the feeding ecology of an endangered primate in Vietnam, its scope is much broader. Within this research is the attention to international cooperation and conservation efforts. This is an opportunity to foster a full and equal partnership between researchers, conservationists, local peoples, and park staff. This is a form of applied anthropological work that, while focused in the forest, is situated within the cultural values, economics, and history of Vietnam. This research will continue to address a number of questions surrounding primate ecology and conservation. The feeding and nutritional ecology of a "specialized" feeder will be investigated in detail. These data can be used to contribute to our understanding of the natural history and adaptive radiation of the colobines in Southeast Asia. With these data, we can begin to understand the comparisons that have been made between African and Asian colobines and evaluate their validity. The similarities or differences noted are important for understanding the evolutionary history of these primates. Adding a landscape ecological perspective refocuses our attention from short studies over a relatively small homogeneous area to larger spatial and temporal areas with the inclusion of humans on the landscape (Turner et al., 2001). Vietnamese primates can be conserved but it will require dedicated work in the form of international cooperation. Levin (2000) noted that humans continue to alter the landscape without regard to global biodiversity loss. He stated that the only way to transform this dilemma is to change the behavioral and social norms that influence this destruction. He concluded that the path to sustainability will only be realized when humans accept their place within nature and change their behaviors for the betterment of the global commons. I can think of no better discipline than anthropology to research and understand the ecological consequences of action and non-action, devise solutions, and then work with local peoples to be able to integrate these ideas into practice.

Vietnamese primates can be conserved. It will take hard, dedicated work in the form of international cooperation. The creation and continuation of groups like the Creating Protected Areas for Resource Conservation using Landscape Ecology (PARC) have implications for the conservation of biodiversity in Vietnam. Levin articulated the need for understanding scale for the sustainability of conservation programs.

> "One of the greatest challenges facing humanity involves the distinct scales of environmental change and human response. As we transform our landscapes through a variety of patterns of exploitation, changes emerge in terms of global biodiversity loss and the status of our oceans and atmosphere. Yet, the curse of scale means that these global changes are sometimes slow to make themselves clear and that humans are even slower to adjust their own behaviors accordingly. The fundamental problem, of course, is that we live in a global commons, in which the scale of the problem overwhelms us in terms of our ability to make a difference. The path to solving this dilemma involves changing patterns of behavior and social norms that influence them; but such norms also typically change only on those longer time scales. Sustainability in the new millennium will depend on our ability to affect with sufficient dispatch the cultural norms and legal instruments that govern individual behaviors in the global commons (Levin, 2000: 504)."

The conservation of primates in Vietnam can be evaluated directly through many of the key points in anthropological primatology and conservation biology. As Wu and Hobbs (2002) concluded, the continued incorporation of humans into ecology will require the broad perspectives available with interdisciplinary work. Through cooperation along many lines we can seek to lessen our impact on landscape change and conserve these endangered evolutionary kin.

2.14 Summary

In this chapter the historical background for this research was discussed. This helps to situate the research within the literature and gives a better understanding of Cat Tien National Park and some conservation actions in Vietnam. Next I will detail the methods and materials used to conduct this research.

Table 2.2: Primates of Vietnam

Taxa	Common Name	Conservation Status
Strepsirrhini		
Loridae		
Nycticebus pygmaeus	pygmy slow loris	VU
Nycticebus bengalensis	Bengal slow loris	VU
Haplorrhini		
Cercopithecidae		
Colobinae		
Trachypithecus crespusculus[1]	grey langur	EN
Trachypithecus delacouri	Delacour's langur	CR
Trachypithecus francoisi	Francois's langur	EN
Trachypithecus germaini	Indochinese silvered langur	EN
Trachypithecus margarita[2]	Annamese silvered langur	EN
Trachypithecus laotum hatinensis	Hatinh langur	VU
Trachypithecus laotum ebenus	Black langur	VU
Trachypithecus poliocephalus poliocephalus	Cat Ba langur	CR
Pygathrix cinerea	grey-shanked douc	CR
Pygathrix nemaeus	red-shanked douc	EN
Pygathrix nigripes	black-shanked douc	EN
Rhinopithecus avunculus	Tonkin snub-nosed monkey	CR
Cercopithecinae		
Macaca arctoides	stump-tailed macaque	VU
Macaca assamensis assamensis	Assamese macaque	NT
Macaca fascicularis fascicularis	long-tailed macaque	LC
Macaca fascicularis condorensis	Con Song long-tailed macaque	VU
Macaca leonina	northern pig-tailed macaque	VU
Macaca mulatta	rhesus macaque	LC
Hylobatidae		
Nomascus annamensis[3]	northern buff-cheeked gibbon	NE
Nomascus concolor	black-crested gibbon	CR
Nomascus gabriellae	yellow-cheeked crested gibbon	EN
Nomascus leucogenys	northern white-cheeked gibbon	CR
Nomascus nasutus	Cao-vit crested gibbon	CR
Nomascus siki	southern white-cheeked gibbon	EN

[1]Included in *Trachypithecus phayrei* by IUCN, [2]Included in *Trachypithecus germaini* by IUCN, and [3]Recently named species yet to be listed by IUCN

CR = critically endangered, EN = endangered, VU = vulnerable, NT = near threatened, LC = least concern, NE = not evaluated

CHAPTER 3: METHODS AND MATERIALS

3.1 Introduction

This chapter outlines the general methods and materials that were used in this study. The information covered includes a description of the study site, phenology, soil, weather, behavioral observations, collection of plant materials, and parasite methodology.

3.2 Study site

Cat Tien National Park (11°21'N- 11°48'N, 107°10'E -107°34'E) was designated a protected area in 1978 and upgraded to a national park in 1992 (Pham Nhat et al., 2001; Birdlife, 2004). The park is 150 km north of Ho Chi Minh City and spreads across three provinces: Lam Dong, Dong Nai, and Binh Phuoc. As outlined by Birdlife (2004), the national park was created under a series of government decrees, starting with No. 360/TTg on 7 July 1978 and included 35,000 ha in the Southern Cat Tien region (Nam Cat Tien). Tay Cat Tien was created in 9 Aug 1986 by decree No. 194/CT (MARD, 1997) and has 10,000 ha. The Cat Loc sector was decreed by Official Letter No. 686/ CV on 23 Oct 1992 after the rediscovery of the Javan Rhino in this area (Schaller et al., 1990). These three areas were joined together to establish Cat Tien National Park on 13 January 1992 by Decision No. 08/CT. My work was concentrated in the southern Cat Tien Sector.

3.3 Forest Structure

In this section, I detail the methods used for measurement and quantification of the forest structure in Cat Tien NP. Setting up and monitoring of three phenology transects was the foundation for this work. In addition to monitoring the plant materials growing along the transects, I also recorded standard forest measurements (diameter at breast height [DBH], tree height, and mortality), canopy cover, soil chemical composition, and finally local weather.

3.3.1 Phenology transects

Three phenology transects were established in the Nam Cat Tien sector to better understand the forest leaf and fruit abundance. A map of Cat Tien National Park was gridded and numbered in order to use a random number generator to establish a stratified random sample. After discussing the plan with the park management, it was decided that this work would only be conducted within the southern park sector (Nam Cat Tien). This decision ended up being extremely beneficial as it allowed more work to be completed with less travel time and fewer permission issues. The map was refocused on the southern sector and three areas were selected at random. Once in the areas, the compass degree was again randomly selected and a 500 m long by 4 m transect was established. One transect was 250 m by 8 m because the forest ended at shortly after 250 m, yielding a transect that contained the same amount of area as the two 500 m transects. All trees greater than 10 cm DBH located within transects were included in the analysis, and all trees were measured for DBH, height, canopy diameter, ratio of leaf flush (new leaves, mature leaves, senescent leaves), ratio of fruit, ratio of flowers, and any other pertinent information (White and Edwards, 2000). The method to determine the ratios of each of these measures was a 4-point scale: the canopy of the tree is divided into four quarters and each quarter is assessed to see how many of each type of leaf or fruit or flower is present (White and Edwards, 2000). If the tree is full of new leaves, then it would receive a score of '4,' if the tree has only half coverage of new leaves it would receive a score of '2.' It is possible to subdivide the units to '.5' indicating that only 1/8 of the tree has that particular element present. These transects were monitored bi-monthly for 14 months and monthly for 11 months for a total of 25 months of monitoring.

3.3.2 Phenology measurement

3.3.2.1 DBH

Diameter at breast height (DBH) is measured at 1.2 m from the ground on the trunk of the tree (Chapman et al., 1992). All trees greater than 10cm DBH were included in the study. A DBH of 10 cm was used as a proxy for the age of the tree and the ability to produce fruit (Chapman et al., 1992, Ganzhorn, 2003).

3.3.2.2 Tree Height

To take tree height measurements, we followed the method of using a range finder to accurately measure the distance to the top of the tree described by Ganzhorn (2003). When it was not possible to use the rangefinder, these data were complimented by visually estimating the tree height with the help of a field assistant using a fixed 2 m object as a visual reference.

3.3.2.3 Mortality

Mortality was measured along the transects while conducting the phenology analysis. Each of the three transects experienced some mortality of trees originally recruited into the study. These trees were noted as dying and ignored for future phenology counts.

3.3.3 Canopy Cover

Canopy cover was measured along the transects at 10m intervals using a GRS densitometer (following Stumpf, 1993). The densitometer allowed for rapid measurement of the percentage of canopy closure or openness to determine if the sample was more open (more sky visible) or more closed (more leaf visible).

3.3.4 Soil

3.3.4.1 Field methods

Soil was collected once in the dry season (February 2010) and once in the wet season (May 2009) in this research. The purpose of the soil collection was to test for the nitrogen, potassium, and phosphorous content of the soils in different seasons. The soils were collected along the phenology transect line at intervals of 20 m. The soils were dug to a depth of 15 cm and then collected in specimen jars. The jars were brought back to the headquarters area and dried in the room to prevent mold growth on the samples. Once the samples were dried, they were sieved out using a colander (3 mm) to a uniform grain size.

3.3.4.2 Laboratory methods and analysis

Once the samples were prepared, the directions on the LaMotte NPK kit (LeMotte 3-5880) were followed using distilled water. First was the extraction phase; the extraction tube was filed with 30 ml of distilled water and then two Floc-Ex Tablets (5504 A) were mixed into the tube. Once the tablets had fully disintegrated, a teaspoon of the soil sample was added to the extraction tube and shaken for one minute. The soil was allowed to settle on the bottom of the extraction tube. The sample was then tested for nitrogen, phosphorus, and potassium content.

The nitrogen sample was prepared with 10 ml of the clear liquid sampled from extraction tube and placed into a separate test tube. One nitrogen tablet (Nitrate WR CTA Tablet- 3703A) was added to the sample and mixed until dissolved. The sample then rested for five minutes before being compared to the provided color chart to identify low, medium, or high concentrations of nitrogen.

The phosphorus sample was prepared with 3 ml of the extracted solution placed into a test tube. An additional 7 ml of distilled water was put into the test tube. A phosphorus tablet

(5422 A) was dissolved into the test tube and allowed to rest for 5 minutes. The sample was then compared to the color chart to identify low, medium, or high concentrations of phosphorus.

The potassium sample was prepared with 10 ml of solution from the extraction tube. A potassium tablet (5424 A) was dissolved in the test tube. The test tube was immediately compared to the potassium color chart to assess the cloudiness of the solution. The cloudiness identified low, medium, or high concentrations of potassium in the sample. These tests provided baseline data for future soil analysis studies.

The soil measurements from this analysis are listed in three categories: high, medium, and low (Appendix 1). Appendix 1 lists the amounts of chemical concentrations per category. These chemical standards were provided by the manufacture of the text applications (www.LaMotte.com). The concentrations for the soil nutrients are listed as kilograms per kilometer squared. The soils were sampled at a depth of 15 cm from the surface as prescribed by the manufacturer. The concentrations are listed in kilograms at a depth of 15.24 cm per square kilometer.

3.3.5 Weather

3.3.5.1 Weather station

A Davis Vantage Pro 2 wireless weather station was used to collect data from November 2008 until February 2011. The weather station was linked to a computer using Weatherlink software. Data collected included: temperature, rainfall, humidity, relative humidity, wind speed and direction, and barometric pressure. The weather data were collected at intervals of 10 minutes. While the weather station functioned well thorough most of the study, a few unresolved issues during the second rainy season, interrupted some of the data collection for rainfall totals.

3.3.5.2 Comparison of weather data to nearby stations

In addition to data collected at Cat Tien NP, weather data was downloaded from the closest government run weather station in Dong Phu, Binh Phuoc Province. This weather station is located northwest of Cat Tien NP and is similar in altitude. This comparison of weather data across a greater temporal range confirms that weather collected represents a normal weather pattern and not an unusual year.

3.4 Daily follows and behavioral observations

Daily follows began shortly after sunrise and were conducted in the forest block directly behind the National Park Headquarters compound. This is an area of mixed habitat with both deciduous and evergreen trees. The area is also seasonally flooded and has very slight topographic variation. There is a 5 km trail that was once used by the tourist staff of the park to guide tourists through the park, but these trails have fallen out of favor for forest travel. Other researchers continue to use and maintain the trails and they often vary and deviate based on seasonal flooding and tree fall. This area contains at least 5 primate species (*Pygathrix nigripes*, *Nomascus gabriellae*, *Macaca nemestrina*, *Trachypithecus germaini*, and *Nycticebus pygmaeus*).

The most effective method for contacting the groups of doucs in the morning was to walk along the trails as silently as possible because, unlike the golden-cheeked gibbons in the forest, the black-shanked douc do not have a loud morning call to alert group status or location. Most vocalizations are very quiet and seem to be contact calls between group members, so it was best to quietly walk the forest trails and listen for sounds of movement in the canopy. This movement in the canopy was usually indicative of either locomotion behaviors (jumping or scrambling through the canopy) or feeding behaviors. The animals could often be contacted in areas where they had been seen the day before, but I was not always successful in locating them using this approach.

When the groups were found, both focal animal and scan sampling were used to collect behavioral data (Altmann, 1974). While I was not able to identify specific individuals, I could use group composition to determine when different groups were contacted on the same day. Group composition was determined by age class (Adult, juvenile, infant) when deciding if it was the same groups contacted before (e.g. Group A= 1 adult male, 1 adult female, 2 juveniles, 1 infant). A GPS coordinate was taken when animals were contacted and the date, time, distance from observer, location in canopy, and location of other neighbors was noted as well. All behaviors were recorded at one minute intervals unless the animal engaged in the same behavior for longer than 15 minutes (sleeping/ resting), then the time between intervals increased to 5-minute intervals. Categories of behaviors recorded were feeding, moving, resting, looking (vigilance), and sleeping.

3.5 Feeding Items

The feeding items were recorded and trees were tagged using biodegradable flagging tape. The location of the trees identified by a GPS and this information along with the time, date, and a number were written on the tape marking the tree. Marking the trees made future identification and marking of revisiting animals to the trees easier. Photographs of the trees were also taken and included the bark, leaves, cambium, and fruit and flowers when available. At a later date, park staff later provided taxonomic identification of the trees to species, when they were able, or to family for many of the trees.

The phases of leaves or fruits were recorded for all feeding items. Then the items were collected, photographed, and brought back to the headquarters to be prepared for chemical testing.

3.6 Leaf collection

All leaves were collected from the trees the animals were observed feeding in. Leaves of the same phase/ age were collected to closely mirror what the animals were selecting to eat. They were harvested from the trees using multiple methods: climbing into the trees to retrieve leaves, using a pruning saw to cut branches down, and knocking leaves out of the tree using a weighted throw bag. The leaves were set out to air-dry by a fan and were stirred throughout the day in order to have consistent drying and to inhibit mold formation. The sample size for eaten leaves is 20 and for non-eaten leaves is 11.

3.7 Plant chemistry

All chemical analyses were conducted at the Nong Lam University Research Center for Environmental Technology and Natural Resource Management in Ho Chi Minh City, Vietnam. Test included protein, tannin, crude fiber, potassium, sodium, iron, manganese, copper, and zinc (Table 3.1).

Chemical	Test
Protein	AOAC 987.04 (1997)
Tanin	AOAC & TC (2000)
Crude Fiber	AOAC 973.18C (1990)
Calcium	TCVN 6496 (2009)
Potassium	AOAC & TC (2000)
Iron	AOAC & TC (2000)
Sodium	AOAC 969.23 (2005)
Manganese	ACIAR AAS 012 (2007)
Copper	ACIAR AAS 007 (2007)
Zinc	ACIAR AAS 015 (2007)

Table 3.1 Chemical tests conducted by Nong Lam University, Ho Chi Minh City, Vietnam.

3.10 Parasite methodology

Fecal samples for parasite analysis were collected in Cat Tien NP during May 2012. The laboratory analyses were conducted at the University of Colorado in May of 2013 with the assistance of Dr. James Loudon, Mr. Robert Adams, and Ms. Kelsey Robb.

3.10.1 Field Collection

Collection of fecal samples for parasite analysis is both an effective and accurate method to understand parasite taxonomy and loads for a focal animal (Greiner and McIntosh, 2009). During this phase of research, I continued to follow the focal and group scanning methods of Altmann (1974) in the same location behind the headquarters were the previous field observations had been made. The samples were collected following the protocols of Ancrenaz et al. (2003): all samples were collected fresh, and time, date, and location were taken by GPS. All samples were stored in ParaPak 10% buffered formalin containers for transport back to the United States for analysis at the University of Colorado Boulder. The 10% buffered formalin preserves the parasites in the fecal sample while inactivating any infectious agents (Zajac and Conboy, 2006).

3.10.2 Laboratory Analyses

Two methods were used on each sample: the direct smear procedure and the floatation procedure.

Direct Smear Procedure

The direct smear procedure takes a small amount of the fecal sample and smears it directly onto a microscope slide. This method can be very effective at finding parasites during certain life stages, but can under sample and miss other life stages (Greiner and McIntosh, 2009). After the application of the fecal material to the slide, a drop of distilled water is placed on the sample, which is then covered with a cover slide. The slide is scanned at 10x power and viewed in a systematic manner to ensure that the entire contents of the slide are examined. If a parasite or suspected parasite is noted, the object is observed under 40x power to verify that it is actually a parasite and not a pseudoparasite (Zajac and Conboy, 2006). When parasites were found, they were recorded in a log and some were photographed for later reference or identification.

Floatation Procedure

Greiner and McIntosh (2009) noted that the floatation procedure is a good method for identifying nematode and cestode eggs, protozoan cysts, and nematode larvae. Following Greiner and McIntosh (2009), approximately 2 grams of the fecal sample were placed in a centrifuge tube with a sodium nitrate solution and mixed thoroughly to break up the material. The tube is spun in a centrifuge for 5 minutes then removed and topped off with the sodium nitrate solution until a meniscus forms along the top of the tube. A cover slide is placed on top of the meniscus and allowed to set up for 10 minutes. This cover slip is removed and then systematically scanned on 10x power. If any parasites are observed, they are verified at 40x power, recorded in a log, and then some are photographed for reference or further identification.

3.11 Statistics Software

All statistics used in this dissertation were calculated using the JMP Pro 11.0.0 software package.

3.12 Summary

This chapter reviews the methods and materials used to conduct this research. The study site of Cat Tien National Park was described before discussing methods including: forest structure and phenology, soil chemical measurement, weather data collection, animal observations, leaf material collection and chemical analysis, and parasite protocols. The forest structure of Cat Tien NP will be examined in the next chapter.

CHAPTER 4: THE FOREST STRUCTURE OF CAT TIEN NP

4.1 Introduction

This chapter describes the habitat the doucs encounter in Cat Tien NP, setting the stage for following chapters on the ranging, feeding, and finally the parasite ecology of black shank doucs found in this national park. First, it details the forest structure of Cat Tien NP and discusses the three phenology transects that were set up to sample the forest for overall productivity; the measurements include the forest height, tree DBH, tree basal area, mortality, canopy cover (and changes) and phenology. In addition to these measures, this chapter discusses soil chemistry and weather to give a better understanding of the seasonal and changing environment that the doucs inhabit.

Three transects were randomly set within the southern sector of Cat Tien National Park in June of 2007 (Figure 4.1). For each of the transects, a starting point was selected and the line extended into the forest. All trees greater than or equal to 10 cm DBH located within 2 m to either side of the line (or 4 m to either side for Ben Cu) were included within the sample (n = 306). A number of measurements were taken for each tree: the DBH, tree height, distance from the sample line, crown volume, GPS coordinates, location along the line, species identification if possible, and phenology.

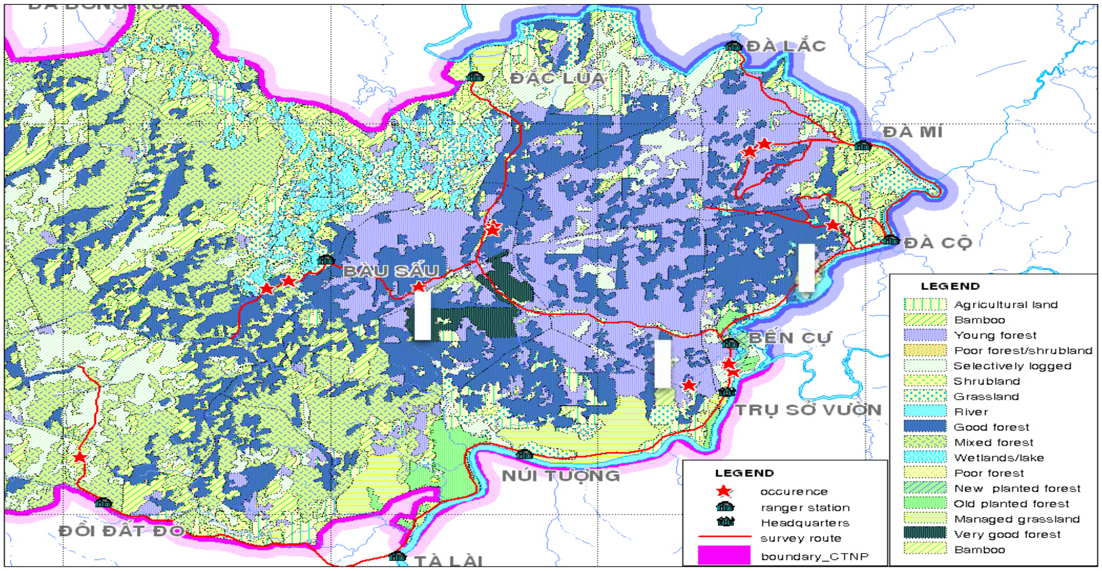

Figure 4.1. Transect locations in Cat Tien NP (Marked with white lines).

The phenology was estimated to describe the tree's current productivity following methods described by White and Edwards (2000). The sample was divided into a number of categories: new leaves, mature leaves, senescent leaves, flowers, immature fruit, mature fruit, and fruit on the ground. All of the trees that were sampled were also marked for future recognition. Canopy cover was also collected at 5m intervals using a vertical densitometer.

The transects were established near the Bau Sau trailhead, near Ben Cu ranger station, and near Da Co ranger station (Figure 4.1). Each transect was proposed to be 500 m by 4 m; ultimately, two of the transects had these measurements, while one of the transects was modified due to the restricted area selected. Regardless, all of the transects measure the same amount of land, 0.2 ha. The use of transects rather than plots provides a better understanding of diversity of forested habitats (Ganzhorn, 2003).

Each of the transects captured a different element of the total forest in Cat Tien NP. The Bau Sau transect crossed a mixed forest type with both young and old growth forest. This transect also had a substantial amount of low-growing groundcover. The Ben Cu transect represents an open evergreen forest type with a mix of old growth and young forest dominated by *Lagerstroemia* trees. As the Da Co transect was situated in a transitional forest zone between bamboo forest and good forest, it covered a number of forest types. This transect had the most variation in landscape and contained the widest samples of trees by DBH.

4.2 Research Aims

Aim 1: How does the forest structure change throughout the year?

Because an understanding of the availability of resources aids in documenting if the doucs are selectively tracking certain resources throughout the year, the phenology transects established at Ben Cu, Da Co, and Bau Sau were monitored for changes in the relative amounts of new leaves, old leaves, senescent leaves, fruits, and flowers. In addition, the canopy structure (open or closed) was assessed each time the transects were walked to note overall levels of forest openness or closure.

Aim 2: How does the soil chemistry differ between seasons?

Soil was collected and analyzed along the three transects for nitrogen, potassium, and phosphorus content. The soil samples were collected in Feb 2009 and May 2010. These collection times allowed for a comparison of soil chemical composition from the dry and the wet season.

Aim 3: How does the weather during the study period compare to typical weather conditions in the region?

A weather station was established at the field site to measure temperature, rainfall, and humidity. In addition to these data, comparisons with long established weather sites in southern Vietnam check the normality of these weather data. The weather station collected data from November 2008 until February 2011.

4.3 Results

4.3.1 Transects

Bau Sau: The Bau Sau transect (500 m by 4 m) is located along the Bau Sau trail leading from the main road of Cat Tien to the lake (Bau Sau translates to “crocodile lake”). The geology of the area is an extinct volcano; as a result, a large amount of volcanic rocks litter the trail system. The area has a series of lakes that make an aquifer system that rises with the rainy season and falls with the dry season. While this transect does not flood during the rainy season, the surrounding area is typically inundated with water. The forest is a mix of deciduous and evergreen trees. Appendix 2 lists the trees located along this transect.

Ben Cu: The Ben Cu transect (250 m x 8 m) is located near the Ben Cu (“old crossing”) rapids in a forest that is predominantly *Lagerstroemia* trees but also includes a mix of deciduous and evergreen trees. This area is surrounded by a series of tributaries of the Dong Nai River and lowland marsh areas and is inundated during the rainy season. Due to the shorter length of this transect, thirteen samples were taken compared to twenty-five in each of the other transects for canopy cover and soil chemistry. Appendix 3 lists the trees located along this transect.

Da Co: The Da Co (“ancient stones”) transect (500 m x 4 m) is located east of the park headquarters along the border of the park next to the Dong Nai river. This transect has the most elevation change of the three, but is still located within the lowland region of the park that floods during the wet season. The forest is comprised of deciduous and evergreen trees and includes some of the larger trees in the Nam Cat Tien sector. Appendix 4 lists the trees located along this transect.

Trees	Bau Sau	Da Co	Ben Cu
Sample size	100	108	98
Mean Height	15.23±3.73	17.86±6.97	15.23±5.06
Mean DBH	21.38±20.2	31.44±32.9	27.2±22.57
Mean Basal Area (m^2)	9.29±7.02	8.65±7.56	11.99±13.65
Mortality Total	4	7	1

Table 4.1. Transect information.

4.3.2 Forest Structure

4.3.2.1 Tree height

As Table 4.1 shows, variation in a number of measures exist across the three transects; these differences suggest interesting possible variation of plant productivity. The Bau Sau transect had 100 trees with an average height of 15.23±3.73 m. The minimum tree height was 8 m and the maximum was 28 m (Figure 4.2). The Ben Cu transect had 98 trees with an average tree height of 15.23±5.06 m, a minimum height of 6 m and a maximum of 28.5 m. The Da Co transect had 108 trees sampled with an average tree height of 17.86±6.97 m, a minimum tree height of 4.5 m and a maximum of 37.5 m.

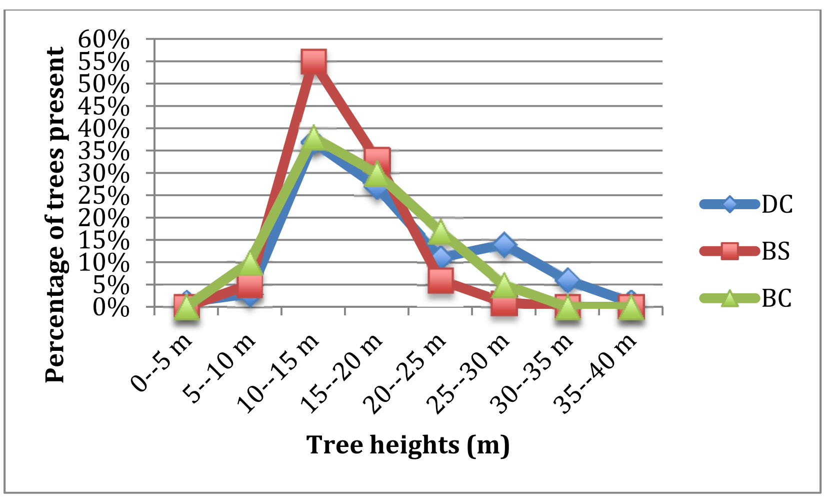

Figure 4.2. Distribution of tree heights (m) by transects. n = 108 (Da Co), 100 (Bau Sau), 98 (Ben Cu).

The distribution of trees varied across the three transects. Only the Da Co transect had a tree less than 5 m in height. The percentages of trees in 5-10 m range were 3% for Da Co, 5% for Bau Sau, and 10% for Ben Cu. In the 10-15 m bracket, Da Co had 37%, Bau Sau had 55%, and Ben Cu had 38%. The Da Co transect had the largest trees of all three transects with 14% in the 25-30 m, 6% in the 30-35 m, and 1% in the 35-40 m range.

The use of a one-way ANOVA to measure variability also showed that the transects differed significantly in height ($F = 12.8666$, $p < 0.0001$). A post-hoc test using Tukey-Kramer HSD revealed that the Da Co and Bau Sau transects differed significantly ($p < 0.0001$) and the Da Co and Ben Cu transect also varied significantly ($p < 0.0019$). The Ben Cu and Bau Sau transects did not vary significantly from each other ($p = 0.3310$).

4.3.2.2 DBH

While Da Co had the largest trees with 6% of trees between 90-200+ cm DBH, the majority of trees on all transects were under 30 cm DBH. In fact, the Da Co transect had the largest mean DBH of all the transects at 31.44±32.9 m, the Ben Cu transect had a mean DBH of 27.2±22.57, and Bau Sau had the smallest mean DBH of 21.38±20.2. Figure 4.3 lists the numbers of trees on each transect by DBH class.

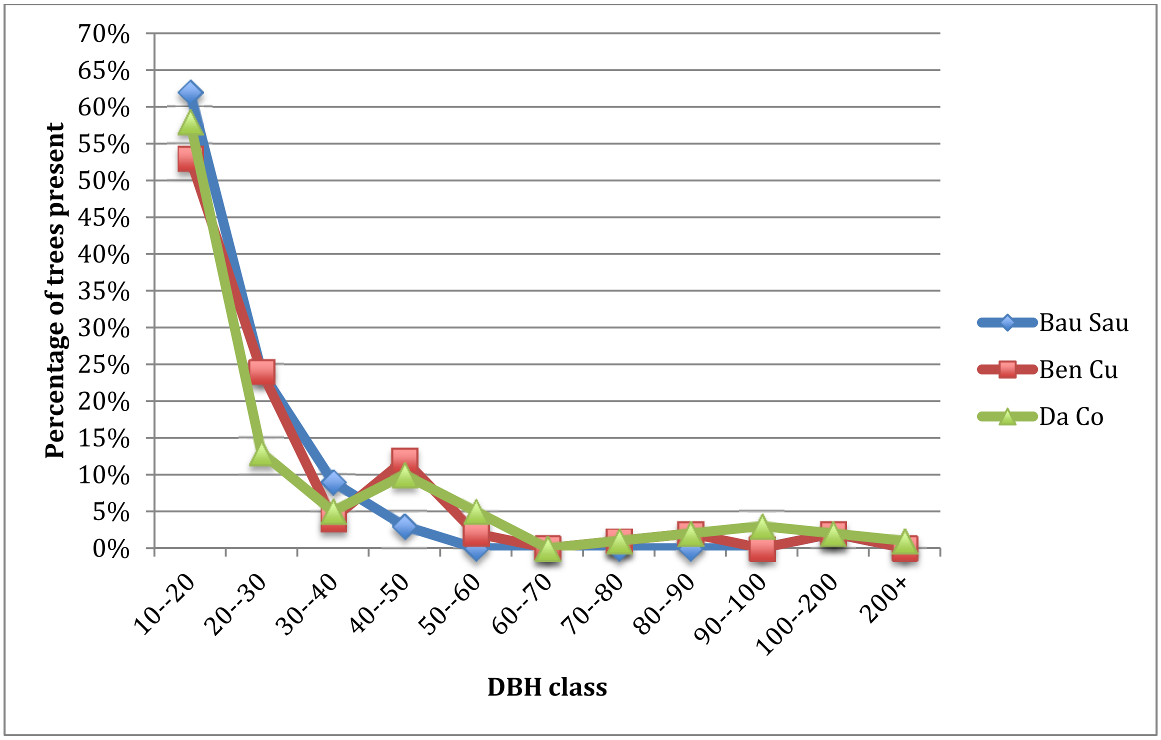

Figure 4.3. Distribution of tree DBH (cm) by transect. n = 108 (Da Co), 100 (Bau Sau), 98 (Ben Cu).

The DBH data were tested using a one-way ANOVA and it was determined that the transects varied significantly by DBH ($F = 3.8868$, $p < 0.0215$). Post hoc testing with the Tukey-Kramer HSD indicated significant variation between Da Co and Bau Sau ($p < 0.0158$), but little variation between either Da Co and Ben Cu ($p = 0.4748$) or Ben Cu and Bau Sau ($p = 0.2593$).

4.3.2.3 Basal Area

Despite the fact that Da Co had the tallest trees and the largest mean DBH, the Ben Cu transect had the largest basal area of tree canopy 11.99±13.65 m^2; in comparison, Bau Sau was 9.29±7.02 m^2 and Da Co was the smallest with 8.65±7.56 m^2. Figure 4.4. shows that 83% of trees on the Da Co transect were less than 15 m^2 in basal area, 80% on the Bau Sau transect, and 77% on the Ben Cu transect.

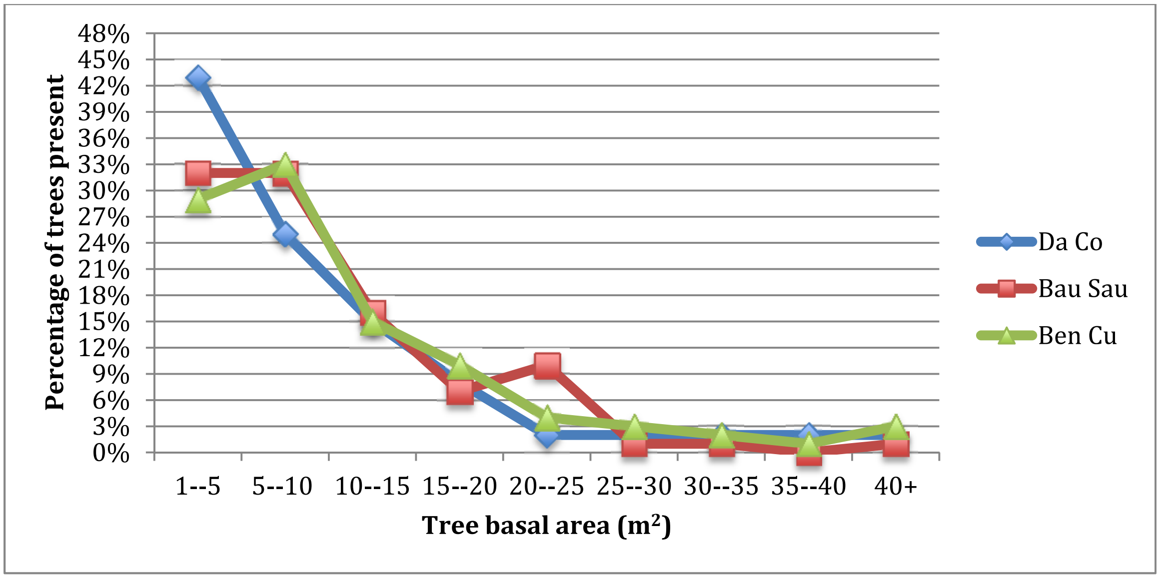

Figure 4.4. Distribution of tree basal area (m^2) by transect. n = 108 (Da Co), 100 (Bau Sau), 98 (Ben Cu).

A one-way ANOVA test was run to compare the transects and found significant differences ($F = 3.2912$, $p < 0.0385$). As with the tests for DBH, a post hoc Tukey-Kramer HSD showed that the Ben Cu and Da Co transects varied significantly ($p < 0.0403$) while both the Ben Cu and Bau Sau transects ($p = 0.1310$) and the Bau Sau and Da Co transects ($p = 0.8841$) did not differ.

4.3.2.4 Canopy Cover

As Figure 4.5 shows, the canopy cover of the transects varied across the seasons. The lowest percentage of canopy cover by survey was 36% for Da Co (Feb. 2009), 42% for Ben Cu (Feb. 2009), and 58% for Bau Sau (Jan. 2009). The highest percentage of canopy cover by survey was 90% for Bau Sau (April 2010), 84% for Da Co (Oct. 2010), and 81% for Ben Cu (June 2009). Canopy cover varied throughout the year with the lowest percentage of canopy cover coming during the dry season when many of the trees lose their leaves.

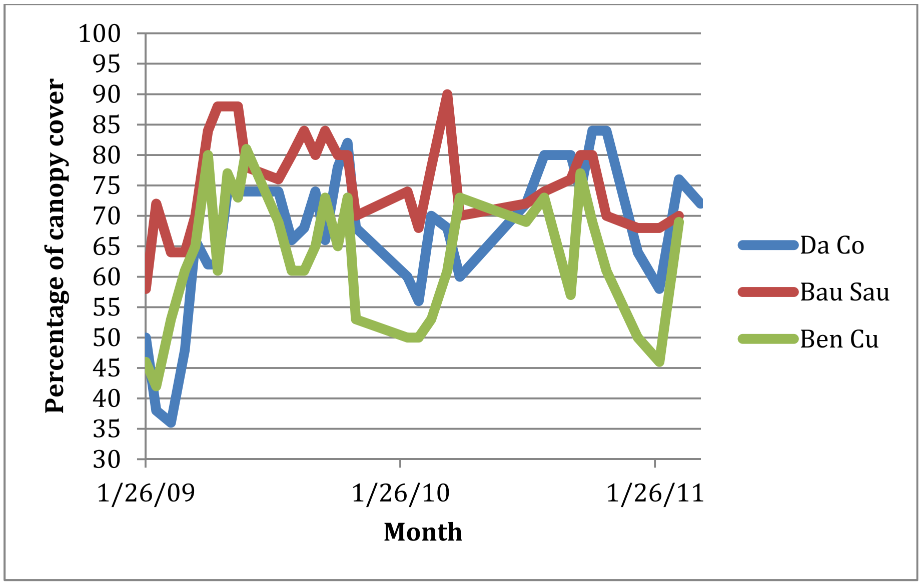

Figure 4.5. Percentage of canopy cover by transect by month.

4.3.3 FOREST PRODUCTIVITY

4.3.3.1 Flowering cycles

As we can see from Figure 4.6, the distribution of flowers present on the three transects changed throughout the study period. Though flowers were not present during every month of the year, the three transects showed no overall difference in flowering ($p = 0.3763$, Wilcoxon test). Over the entire study period, the trees in Da Co flowered for 56% of the time, Ben Cu was in flower for 44%, and Bau Sau had flowers for 36%. Peak production of flowers occurred on the Da Co transect during the month of May 2009 with 14% of trees producing flowers. Ben Cu produced the second greatest percentage of flowers in February 2010 with 11% of trees flowering. Trees in Bau Sau, which did not produce as many flowers as the other two transects, showed the largest percentage in March 2009 at 4%. In 2009, both Da Co and Ben Cu had two separate flowering events; this could be tied to the large amount of rainfall that occurred during that year.

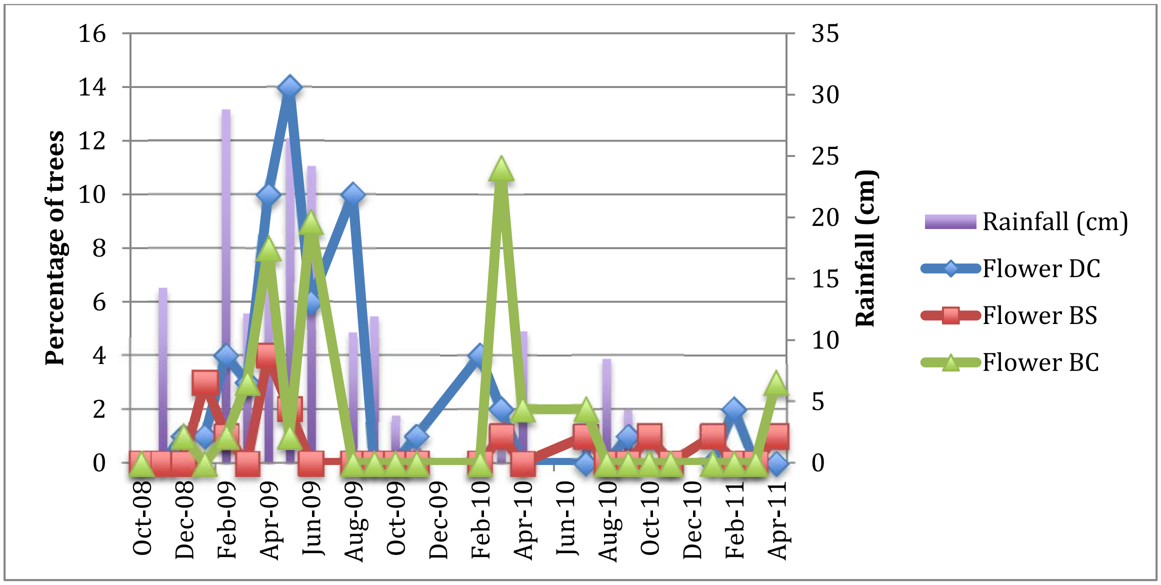

Figure 4.6. Monthly percentage of trees with flowers for all transects including rainfall.

4.3.3.2 Fruiting cycles

Generally, the largest percentage of fruiting followed the month after the transect flowered. Figure 4.7 shows the percentages of trees with fruit for all three transects; for this study, fruit has been compressed into one category rather than being broken into separate phenophases. The three transects did not differ significantly in fruit production ($p = 0.0811$, Wilcoxon test). Fruit was present on the Ben Cu transect 76% of the time, 68% in Bau Sau, and 56% in Da Co. Ben Cu and Da Co both had the largest percentage of fruits available in March 2010 with 10% of the trees in fruit. The Ben Cu transect and Da Co transect were more similar to one another than to Bau Sau in the timing of fruiting.

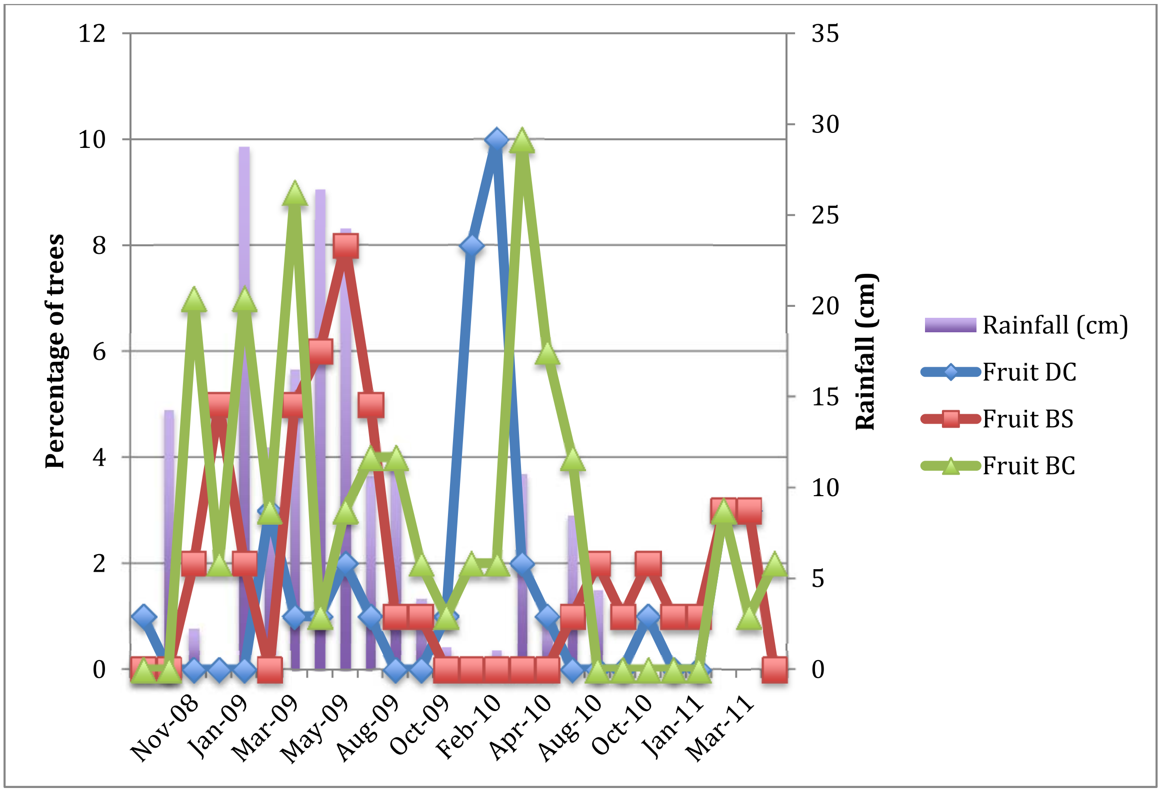

Figure 4.7. Monthly percentage of trees with fruit for all transects including rainfall.

4.3.3.3 Leaf Production cycles

Patterns of leaf flush were very similar across the three transects (p = 0.9481). Figure 4.8 shows the percentage of trees with new leaves for each of the transects. All three transects experienced the greatest amounts of leaf flush starting in March and continuing until June. This pattern is logical: late April / early May is the beginning of the rainy season in Cat Tien NP and the forest is putting on new leaves after the deciduous trees have dropped leaves going into the dry season.

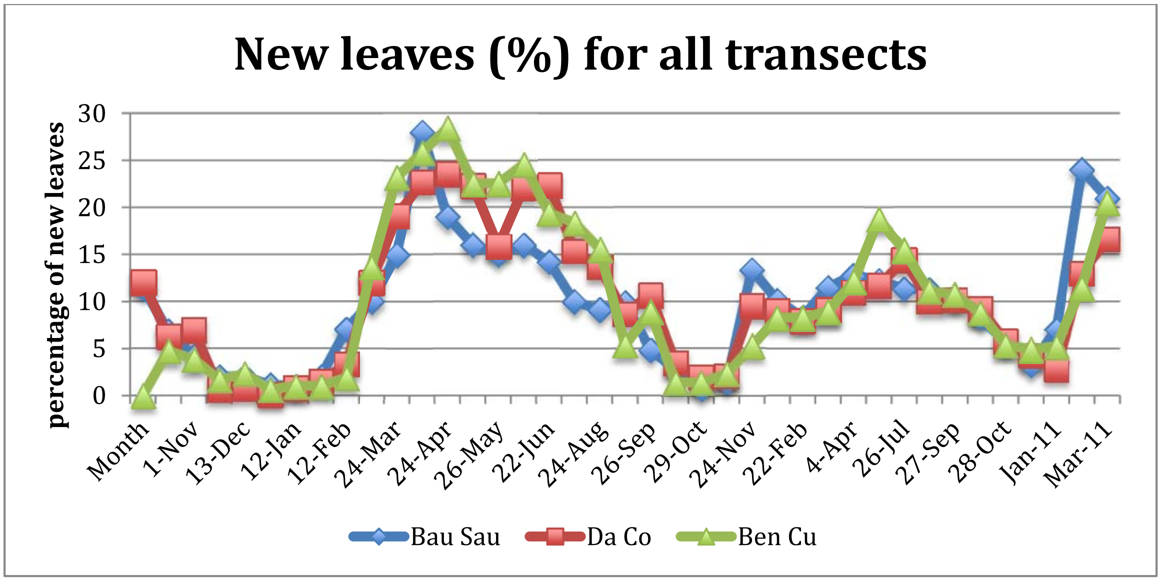

Figure 4.8. Percentage of new leaves for all transects.

Showing the percentage of trees without leaves on the three transects, Figure 4.9 reflects the highly cyclical pattern of leaf loss by the deciduous trees in Cat Tien, and the significant difference in periods of leaf loss ($p < 0.0275$, Wilcoxon test). Ben Cu shows the greatest loss of leaves per transect with up to 51% of the trees having no leaves in March 2009. This pattern repeats with 49% of leaves lost in April 2010. During the same period, Da Co had 42% of trees without leaves (March 2009) and 39% without leaves (March 2010). Bau Sau also showed many trees without leaves in March 2009, but only 29% of trees had no leaves. In 2010 Bau Sau maintained more leaves than the other two transects with only 12% of trees bare of leaves.

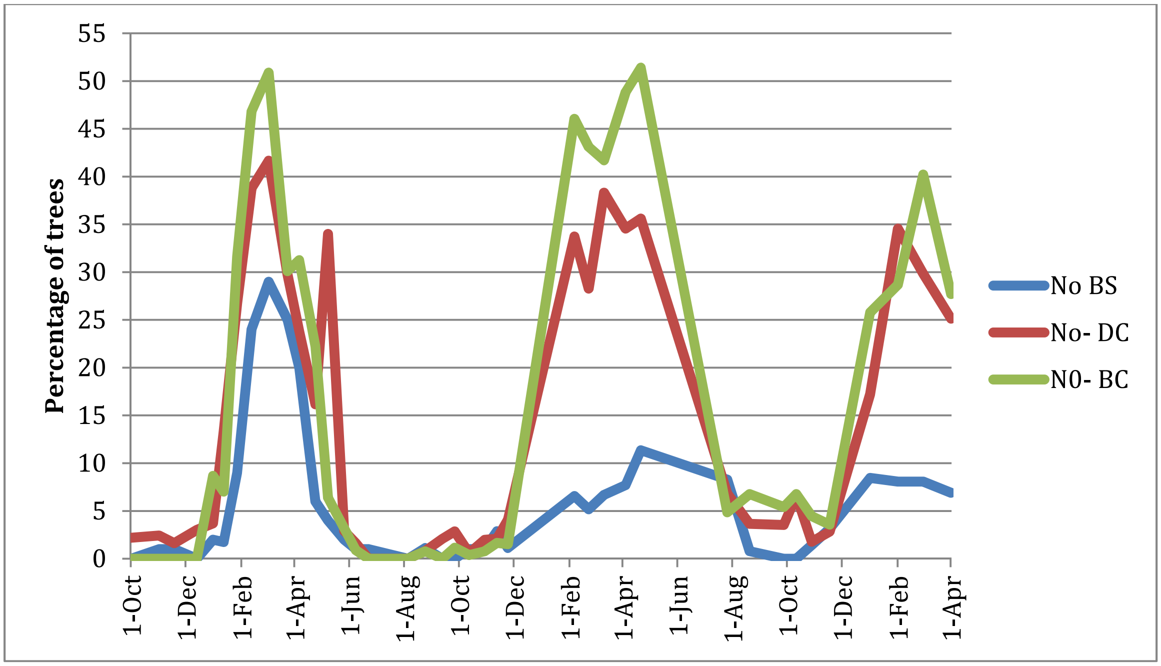

Figure 4.9. Distribution of trees with no leaves on all transects.

4.3.4 Soil

In order to analyze the nutrient compounds in the soil, two different statistics were used. Fisher's Exact test was used because of the small sample sizes that would violate the assumptions of chi square. Fisher's Exact test gives a two sided exact probability. Tests were run at **α** of 0.05. Second, the Lambda Asymmetric C|R was applied as a Measure of Association test. The $\lambda(C|R)$ has a range of $0 \le \lambda \le 1$ and is useful to compare chi squares (and exact tests) of different values independent of n. This test is similar to correlation coefficients with interval and nominal data: if we know the season, then this gives us insight into predicting the variable (soil nutrient level). All of the transects were combined into a single test to be able to evaluate differences between the two seasons for each of the elements tested. One data point per season (wet and dry) was collected to make this comparison, which gives a good initial review of soil chemical variation.

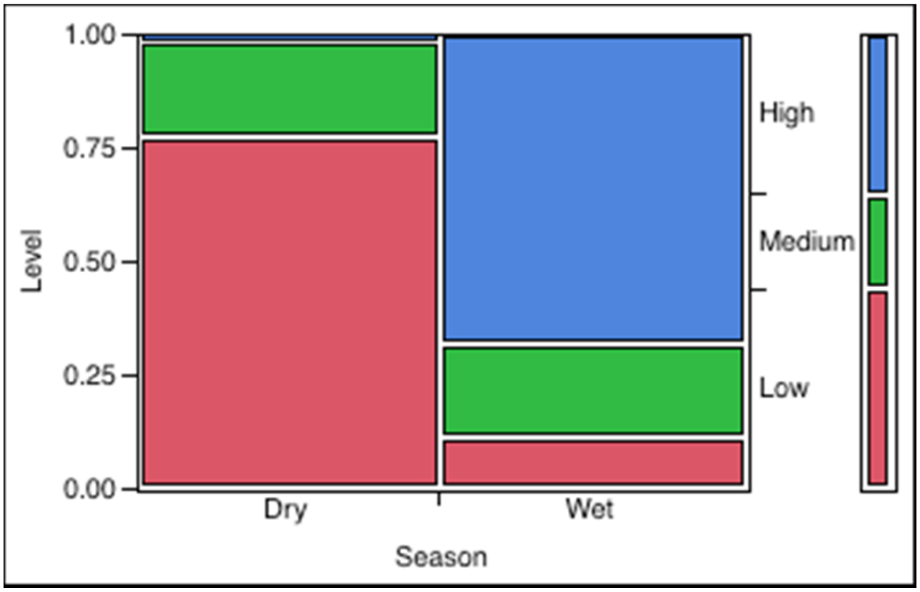

Figure 4.10. All transects combined N level by season: red represents low concentrations, green represents medium concentrations, and blue represents high concentrations.

Figure 4.10 gives the nitrogen content for comparison between the wet and dry season. The seasons showed a significant difference (p <0.0001, Fisher's exact test two sided probability) for N differences between wet and dry seasons. The wet season has a greater percentage of high nitrogen samples, while the dry season has a significantly higher level of low nitrogen samples.

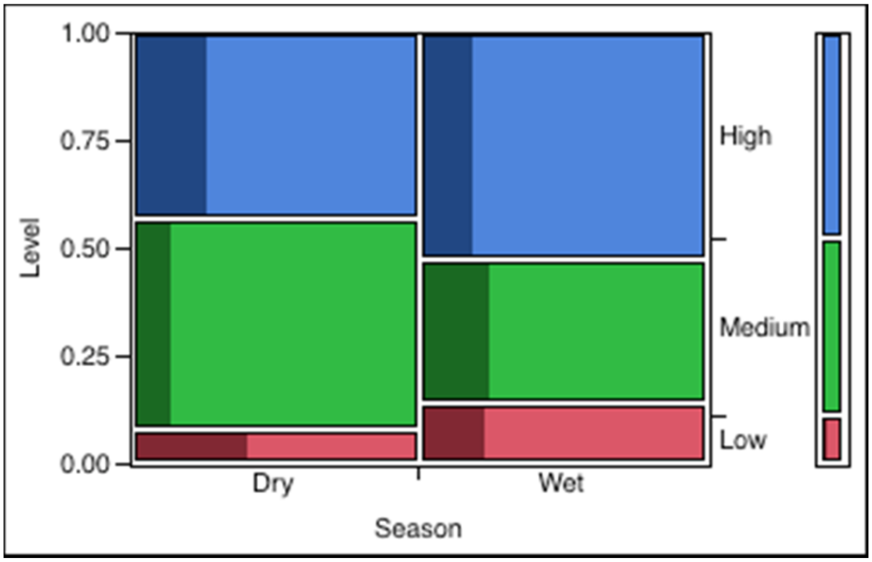

Figure 4.11. All transects combined K level by season: red represents low concentrations, green represents medium concentrations, and blue represents high concentrations.

Figure 4.11 lists the distribution of potassium by season across all transects. This element did not differ significantly between the seasons (p = 0.1561, Fisher's exact test two sided probability).

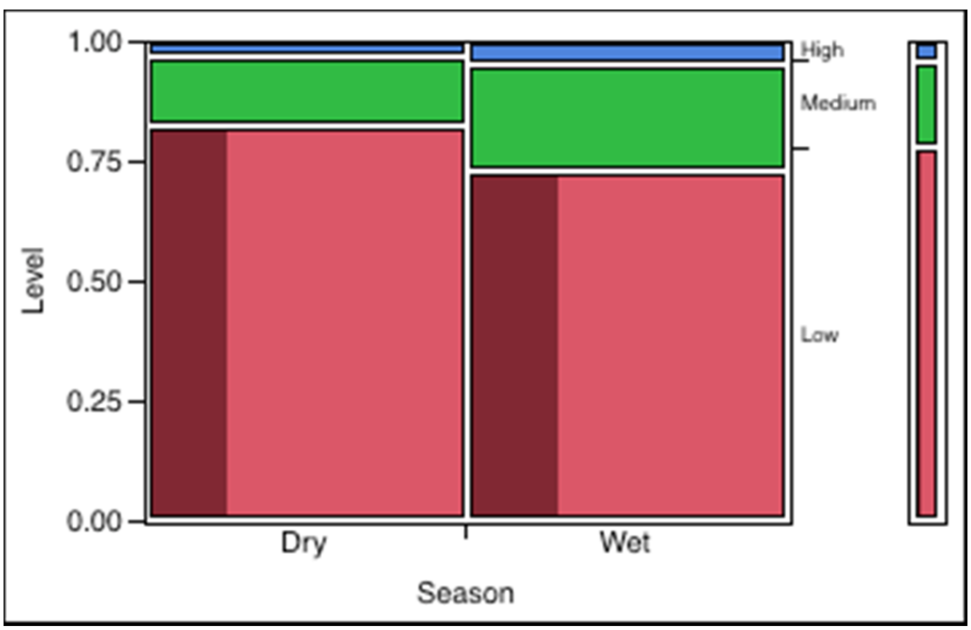

Figure 4.12. All transects combined P level by season: red represents low concentrations, green represents medium concentrations, and blue represents high concentrations.

Figure 4.12 lists the combined potassium samples for both seasons. This element also did not differ significantly between the seasons (p = 0.5266, Fisher's exact test two sided probability).

4.3.5 Weather

The weather was recorded in the park headquarters area with the Davis Vantage Pro 2 wireless weather station. Figure 4.13 lists the monthly maximum temperatures, monthly minimum temperature, and average temperature. In addition, rainfall is set on the secondary axis. The coolest temperatures were recorded in January and the warmest temperatures in April. These data again coincide with the changes of seasons: the coolest temperatures are during the dry season while the hottest temperatures generally occur before the heaviest rains.

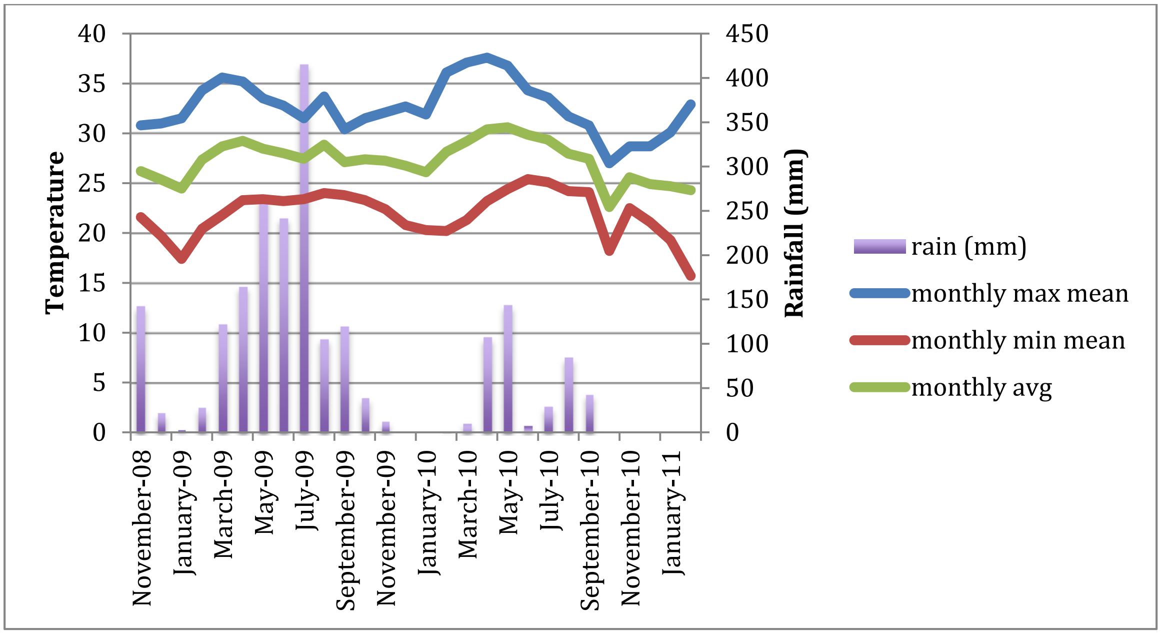

Figure 4.13. Monthly maximum, minimum, average temperatures (°Celsius) and rainfall (mm) in Cat Tien NP.

Figure 4.14 illustrates data from a weather station located in Dong Phu, Binh Phuoc Province, a station located 45 km to the northwest of Cat Tien NP park headquarters and at a very similar altitude. Allowing us to compare the weather data collected in Cat Tien NP to a longer termed weather station, these data show the seasonal trends of this region with a very similar pattern of temperatures and rainfall.

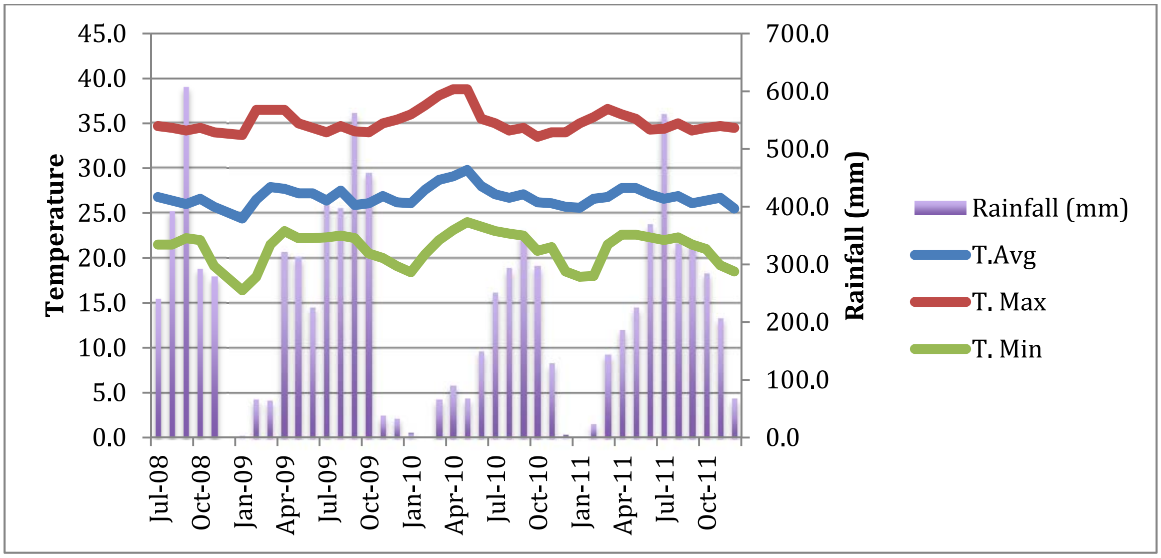

Figure 4.14. Dong Phu weather station.

For a direct comparison of the weather station in Cat Tien and the weather station in Dong Phu for the months of November 2008 till March 2011, Figure 4.15 records the monthly temperature maximums, averages, minimums, and rainfall. While not identical, the weather patterns revealed in this comparison are similar. The data trends are similar and show the seasonal variation in the region.

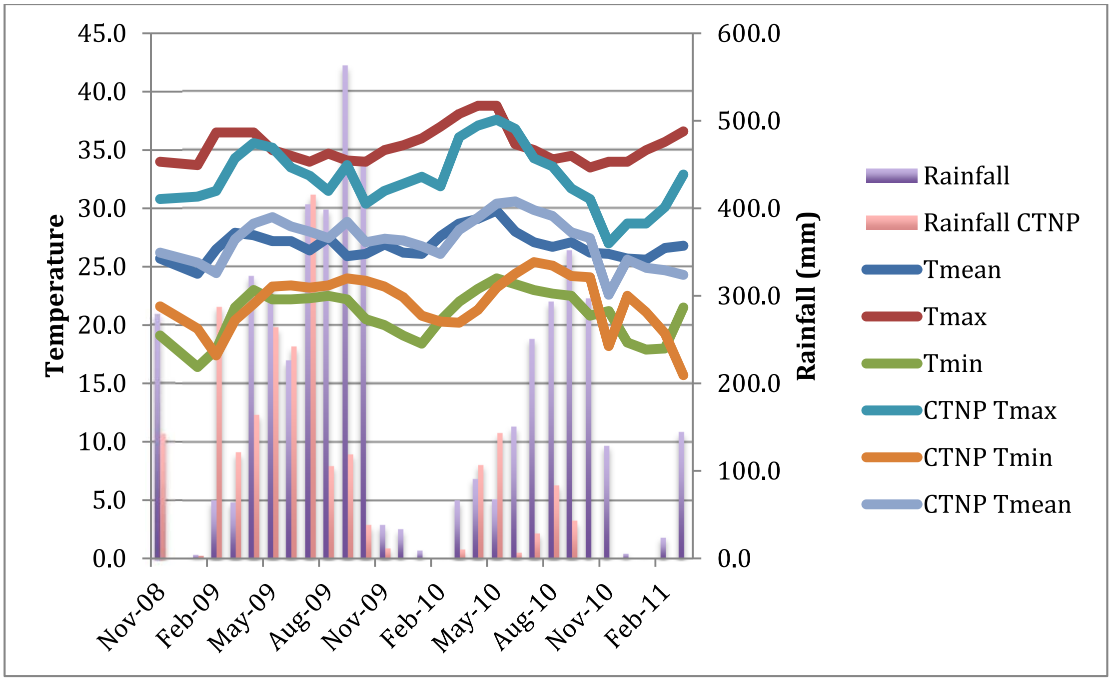

Figure 4.15. Cat Tien NP and Dong Phu weather stations compared.

4.4 Discussion

This chapter began with a series of research aims to understand the forest structure of Cat Tien NP as it applies to the nutritional ecology of the black-shanked douc. As mentioned in the background information, Westing (1971) described Cat Tien NP as having a mosaic of different plant communities; these communities have been influenced by logging, farming, and herbicide damage during the American conflict. Blasco et al. (1996) classified Cat Tien NP as a disturbed semi-evergreen forest at a transitional zone between wet and dry forest types. Blanc et al. (2000) conducted structural and floristic composition plots in Cat Tien NP to describe the successional trends and also concluded that Cat Tien is a mosaic habitat. The doucs are living not only in a seasonal environment, but also in a landscape that is not homogenous in composition. Where they move and when they move to those areas is important for their survival and maintenance as I will explore further in chapter 5.

The results presented here tell a story of a forest that has times of abundance and times when resources are scarce. The forests of Cat Tien NP are not static entities: they are clearly seasonal forests that experience and reflect dry periods and wet periods. Starting in December and peaking in January through April, the forest loses many leaves from the deciduous trees, which coincides with dry season and the coolest temperatures of the year.

4.4.1 Structure

While an understanding of how forests vary is interesting, it is more useful to look at why we study the forests. Where are these animals best suited to live? How do the forests where we find black-shanked doucs vary and what are the habitat implications? A comparison with another landscape these animals inhabit can help to understand one piece to the habitat preferences of black-shanked douc. Rawson (2009) conducted research in the Seima Biodiversity Conservation Area in Cambodia, a semi-deciduous forest that is similar to Cat Tien in forest structure, except that it receives more rainfall per year. Interestingly, even though we have very similar landscapes, we see many more animals in Cambodia than Vietnam. In fact, Pollard et al. (2007) recorded the largest population of black-shanked doucs surveyed to date with over 40,000 animals calculated within the Seima Biodiversity Conservation Area (95% confidence interval- 27,309-66,460); a population that is likely five to 10 times as that of the population of this species in Vietnam. They estimated almost 6,000 groups with a mean group size of seven animals (6.1 – 8.3). A full census of black-shanked douc has never been conducted in Cat Tien NP; the only published report on the population size and density of doucs identified at least 18 groups with a minimum of 109 individuals (Phan Duy Thuc et al., 2005). This study suggests a population number at minimum of 200 animals with at least 25 groups located in the Nam Cat Tien sector.

An important difference between the two areas and between the two countries of Vietnam and Cambodia is human population: while Vietnam is only about twice as large as Cambodia, it has six times the human population—close to 92 million people (CIA factbook, 2013) compared to Cambodia's 15 million people (CIA factbook, 2013). The population densities of the countries are also strikingly different with 275 people/ km^2 in Vietnam compared to 78 people/ km^2 in Cambodia. Though we may not have a direct comparison between population numbers and pressures on natural resources, more people on the landscape unquestionably increases pressures on natural areas. A smaller percentage of land being covered in forest and a smaller population of doucs could all be conceivable outcomes from these human population differences.

The transects located at Bau Sau, Da Co, and Ben Cu varied in tree height, mean DBH, mean basal area, and mortality during this study (Table 4.1). Further, comparing the Cat Tien NP sample with the data Rawson presented on tree heights for the three transects sampled during his study, we find that all of the mean tree heights are similar in range (Table 4.2).

Transect	Bau Sau	Da Co	Ben Cu	Rawson 1	Rawson 2	Rawson 3
Tree height (m)	15.23±3.73	17.86±6.97	15.23±5.06	16.87±6.14	17.71±6.23	14.15±4.15
Mean DBH(cm)	21.38±20.2	31.44±32.9	27.2±22.57	26.42±17.94	25.7±17.63	23.11±17.03

Table 4.2. Comparison of Cat Tien NP forest transects and Rawson (2009).

DBH means varied across all three transects, with Bau Sau having the lowest mean (21.38±20.2), Ben Cu being intermediate (27.2±22.57), and Da Co having the largest mean (31.44±32.9). Perhaps significantly, changes in tree height coincide with changes in DBH. Rawson's (2009) sample is most directly comparable to the Ben Cu transect in mean DBH. These data show similarities in forest habitats simply measuring tree heights and tree DBH.

This study reconfirms that Cat Tien NP is a semi evergreen or mixed deciduous forest (Westing, 1971; Blasco et al., 1996; Blanc et al., 2000; Kenyon, 2007) that is in phases of regeneration after human disturbances. The forest continues to regenerate based on the number of small saplings located along the transects.

4.4.2 Phenology

The timing of resource availability within the environment is important for all animals, whether focusing on fruits, flowers, or leaf material. The findings in this study suggest a connection to the work of Van Schaik et al. (1993), who noted that most tropical forests have seasonal phenological patterns that directly affect the species present in the forests. If seasonal resource tracking is demonstrated by understanding the pattern of availability and selection, then it is possible to better understand how the animals are utilizing the resources available to them.

Comparisons with previous studies in Cat Tien NP continue to yield similar results in phenological patterning. The greatest amount of fruiting along the transects occurred between April and June, which is similar to Kenyon (2007). Kenyon (2007) focused on gibbons for her study, which ate primarily fruit, and doucs did ingest fruits in this study and others (Rawson, 2009; Hoang Minh Duc et al., 2011). Measurement of phenological patterns of forests across multiple years can be important to understanding variation in fruiting regimes as many trees do not produce fruit ever year (Frankie et al., 1974; Leigh et al., 1996; Struhsaker, 1997). These comparisons with Kenyon (2007) allow a greater confidence in the patterns recorded for the fruiting of trees along the transects.

Fruiting levels at Cat Tien were variable by month but averaged very low percentages of trees producing fruit: Da Co (1.52%), Ben Cu (2.92%), and Bau Sau (1.96%). These figures are quite low compared to many other studies in the region: Rawson (2009) in Cambodia recorded

13%, Bartlett (1999) reported a maximum of 9% from Khao Yai NP, and Palombit (1992) in Sumatra reported averages of 7.7%± 4.3. While not in the region, Chapman et al. (2005) reported monthly averages of 3.9% in Kibale NP, Uganda. Comparing the Cat Tien NP sample to Rawson's sample may suggest another reason why the populations vary so greatly. There appears to be a much higher fruit production within the Semia Biodiversity Conservation Area and this may allow for a larger population of black-shanked doucs.

Borchert et al. (2002) related water availability or water stress to patterns of leaf loss and leaf flush, finding that this interplay of environmental factors effects when leaves are lost and gained in seasonal forests. Comparing leaf flush between this study and Kenyon's study in Cat Tien NP, the greatest percentage of new leaf flush on transects during this study did not measure over 30% of leaf material. The overall percentages of new leaves was much higher during Kenyon's (2007) study, with the 'evergreen' transect trees producing over 40% new leaf material. This difference can be attributed to the differences in where the samples were taken, but importantly, the pattern is same for leaf flush and leaf loss throughout the year.

4.4.3 Soil

This study measured soil nutrient availability for the dry season and wet season to assess seasonal variation. The nutrients were measured for high, medium, and low concentrations of nitrogen, potassium, and phosphorus. Nitrogen was the only element to vary significantly between the wet season and the dry season ($p < 0.0001$, Fisher's exact test two sided probability). While phosphorous and potassium did not vary significantly between the seasons, phosphorus measured to be predominantly low in concentration during both seasons, and potassium was fairly equal in high and medium concentrations between the seasons. Low mineral content of certain soils, together with leaching due to high rainfall, may reduce growing conditions for trees, resulting in unpredictable fruiting and poor foliage for folivores; fruit and high quality leaves require more minerals to produce (Oates, 1990; Ganzhorn et al., 1999). This study shows that there is a difference between the seasons with the wet season having higher nitrogen availability. Forests with greater seasonality, soil infertility, or unfavorable rooting conditions often have higher levels of anti-herbivore strategies (chemical) than less seasonal forests (Givnish, 1990). Cat Tien NP has been described as having shallow, ferralitic soils that allow for frequent tree falls (opening of the canopy) and desiccation of the soil during the dry season (Blanc et al., 2000; Kenyon, 2007). Soil conditions have a significant impact on tropical forest vegetation, but a direct causal link between soil chemistry and primate biomass in an area is unlikely to be elucidated (Oates et al., 1990). Furthermore, soil geochemistry might ultimately be responsible for variation in primate abundance in some cases, but likely other factors may be more important (Colinshaw and Dunbar 2000). The data from this study offer a preliminary argument that nitrogen content is greater in the wet season; future research can measure changes in leaf and fruit phytochemicals to see how the chemical availability of nutrients changes between the seasons.

4.5 Summary

This chapter reviewed the structure of the forest in Cat Tien NP. It discussed a number of variables between the three transects of Bau Sau, Da Co, and Ben Cu including: tree height, DBH, basal area, new leaf flush, leaf fall, weather, and finally soil chemical variability. These measures help to define the forest; the next chapter discusses where the doucs and feeding trees were located.

CHAPTER 5: RANGING BEHAVIOR AND FEEDING TREES

5.1 Introduction

Chapter one began with the question: If these are leaf-eating monkeys and the forest is made of trees covered with leaves, why bother to ever move? Building off the previous chapter's description of the forest structure of Cat Tien NP, this chapter focuses on the areas in the forest where the doucs were routinely observed, and, more specifically, on the locations of the particular trees that the doucs fed upon. Chapter four showed the seasonal nature of Cat Tien NP through phenological surveys of three transects: seasonality is vital and the geographical assessments given here are both spatial and temporal in nature, reflective of the forest structure changing throughout the year. Not surprisingly, then, the following data reflects how ranging and feeding tree location vary across the seasons; as Yeager and Kirkpatrick (1998) discussed, a number of ecological factors can influence social structures including abundances of resources and parasites (Chapter seven).

5.2 Research Aims

Aim 1: Identify areas where the Black-shanked douc concentrate their activities.

One of the aspects of this research was to see where the doucs were concentrating their activities in order to better understand what sort of habitat was preferred. The previous chapter described how the forest is not homogeneous in structure, so the question then becomes: how does forest structure affect behavioral patterns?

Aim 2: Identify locations of feeding trees.

Establish the locations of trees where the doucs fed: is there a significant pattern? Cat Tien NP is a deciduous lowland tropical rain forest. Because many of the trees in Cat Tien NP are deciduous, seasonal changes affect the forest canopy structure and thus alter how the doucs interact with the forest. The doucs were always encountered in forest blocks dominated by *Lagerstroemia* trees; however, while the doucs often utilized these trees for moving through the forest, they were never seen feeding off of these deciduous trees.

5.3 Ranging of black-shanked doucs in Cat Tien NP

Figure 5.1. Cat Tien NP aerial view. The Nam Cat Tien sector is bordered on the north, east, and south by the Dong Nai River. The park headquarters is marked with an arrow.

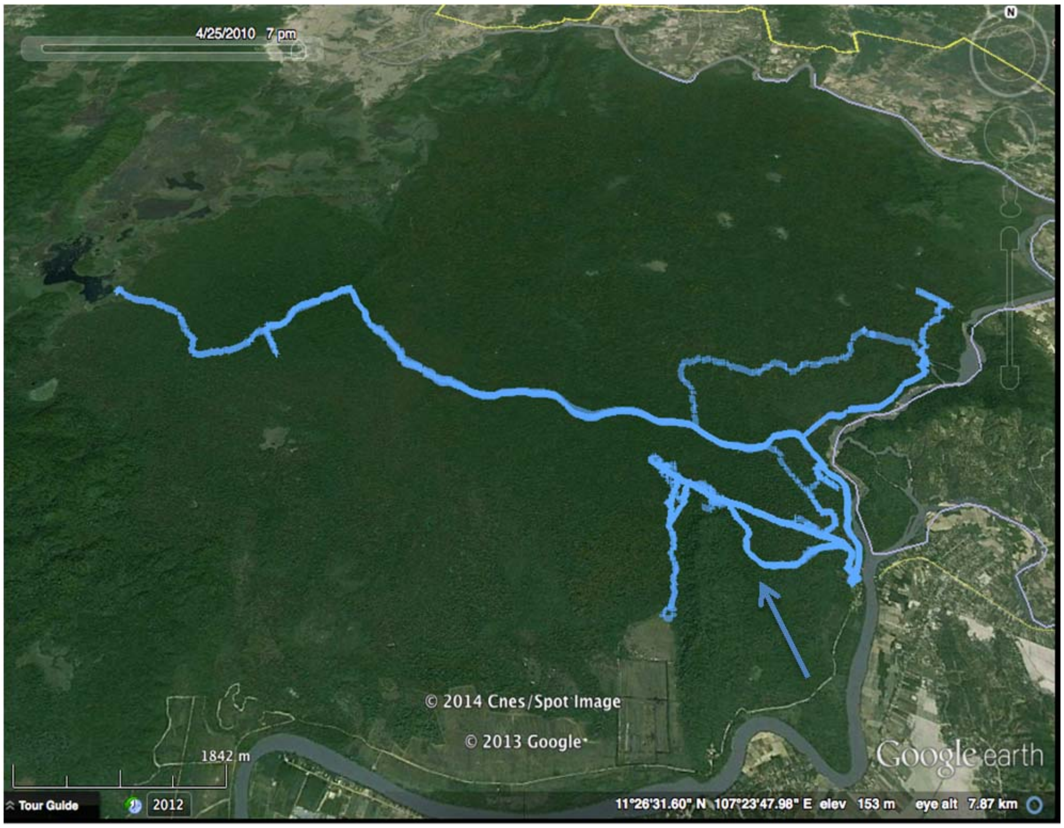

Figure 5.2. Cat Tien NP aerial map of the park headquarter region with GPS tracks overlaid to show the transect areas of Bau Sau to the west, Da Co to the east, Ben Cu to the center, and park headquarter and 5 km research trail marked by the arrow.

Figure 5.1 is an aerial view of the Nam Cat Tien Sector. Figure 5.2 is the Nam Cat Tien sector with GPS tracks overlaid on the image; these tracks include all areas surveyed for primates and transects created for monitoring the phenology. The results presented here for the locations of the black-shanked douc focus on the park headquarters' area. This area is marked on Figure 5.2 with an arrow indicating the center of the 5 km trail that was routinely walked. This area is always a favorable location to encounter the doucs, pig-tailed macaques, and yellow-cheeked gibbons. To explain variation between wet and dry season ranging, Figure 5.3 shows the three transect areas and the headquarters' area of the national park.

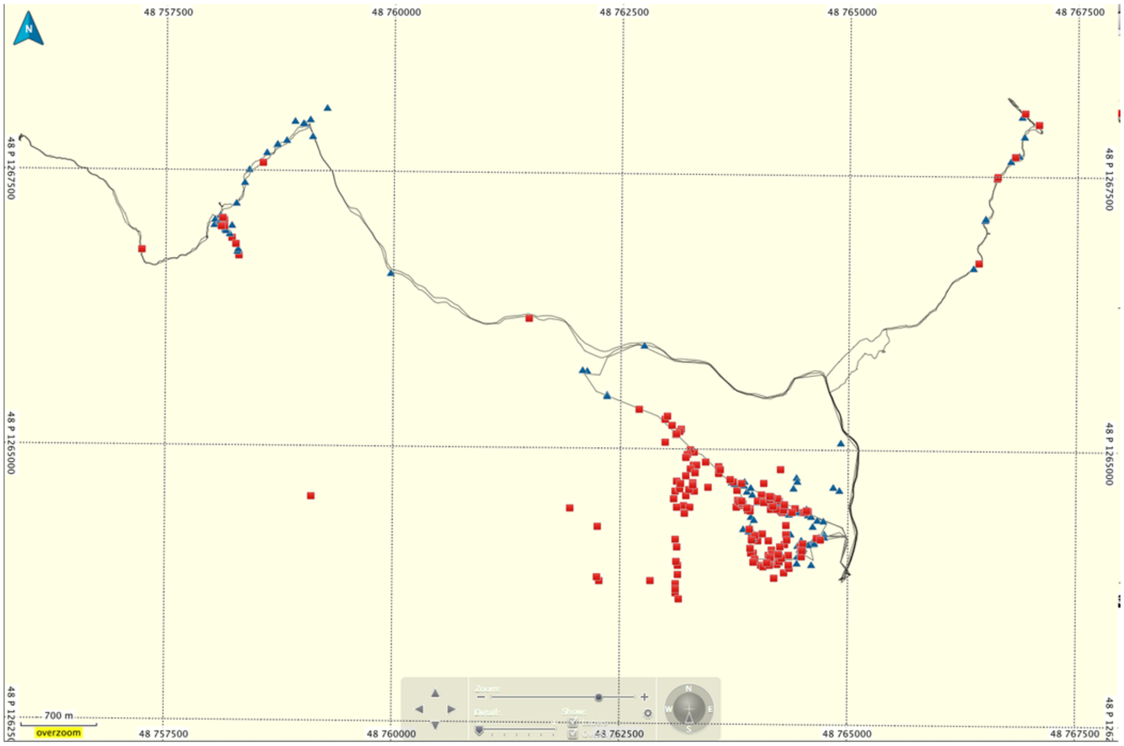

Figure 5.3. All transect areas for wet and dry season occurrences of doucs. Bau Sau transect is the northwest occurrences, Da Co the northeast occurrences and Ben Cu is the south-central occurrences. (Red squares are dry season douc encounters; blue triangles are wet season encounters.)

The Bau Sau and Da Co transect areas show similar concentrations of douc encounters for both the wet and dry season, while the headquarters' area and Ben Cu transect area show greater differences between douc encounters for the wet and dry season.

Figure 5.4 maps the locations of black-shanked doucs encountered during the wet season (n = 112). The doucs decrease their ranging behavior during the wet season compared to the dry season with one occurrence west of UTM 48 762750 and one occurrence north of UTM 48 P1264750. Most of the douc occurrences in the rainy season are concentrated along the south-east section of this trail loop, east of UTM 48 764250. This changes during the dry season when activity shifts to the west of this UTM line.

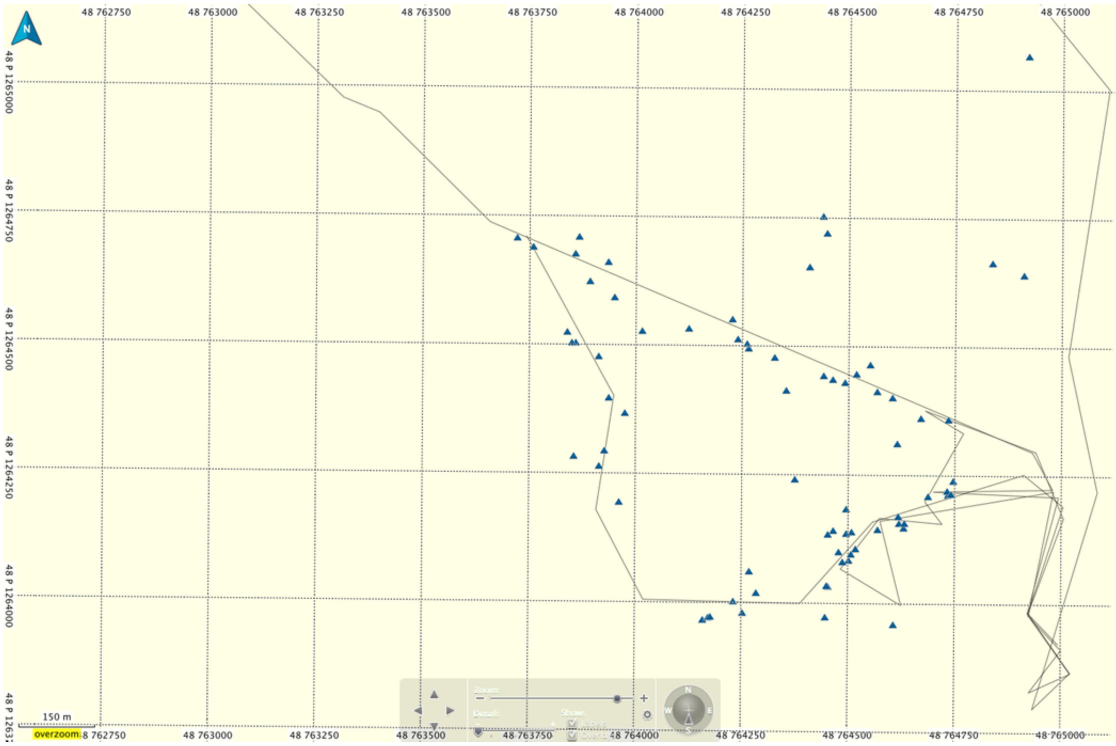

Figure 5.4. Locations of all douc encounters in the wet season.

Figure 5.5 maps the locations of black-shanked doucs encountered during the dry season (n = 165). In the dry season, there are clear indications of occurrences of doucs in the western portion of the study site. These maps do not capture the topography of the area, but the only hill in the southeastern section of Cat Tien NP is located between UTM 48 763500 and UTM 48 763750. The shift to areas dominated by *Afzelia* trees occurs during the dry season, when other trees have not yet begun to regrow their leaves, but *Afzelia* are beginning to bud.

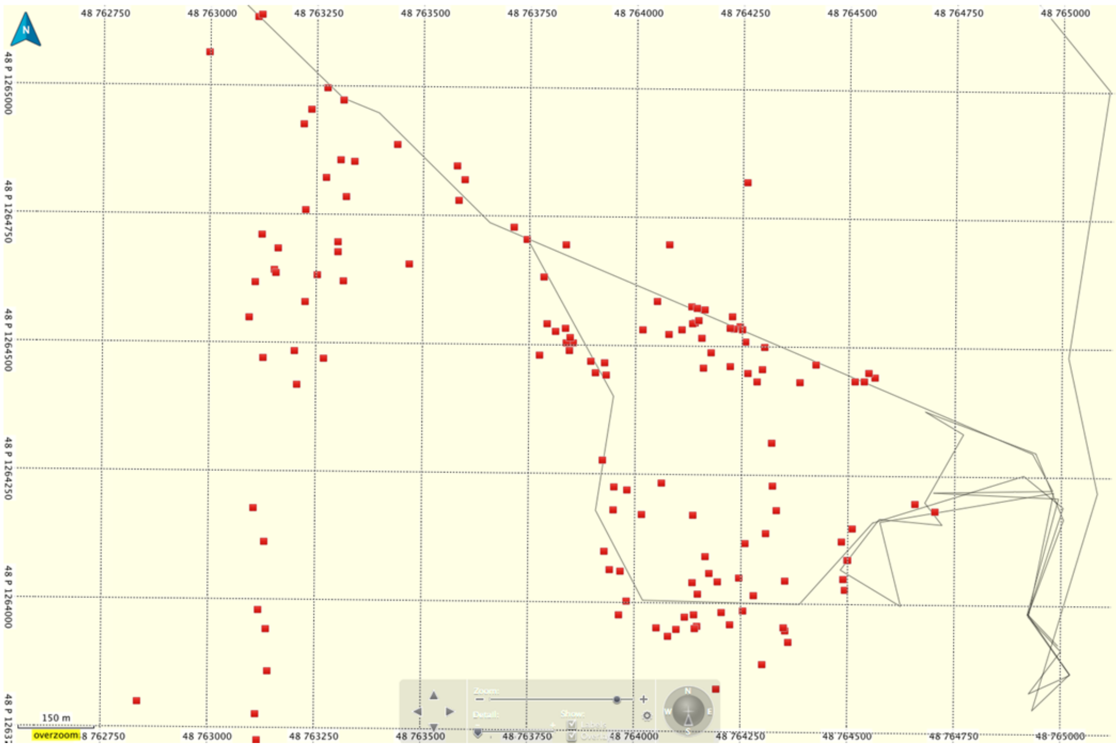

Figure 5.5. Locations of all douc encounters in the dry season.

Figure 5.6 combines the locations of the doucs encountered to clearly illustrate this seasonal shift in habitat use.

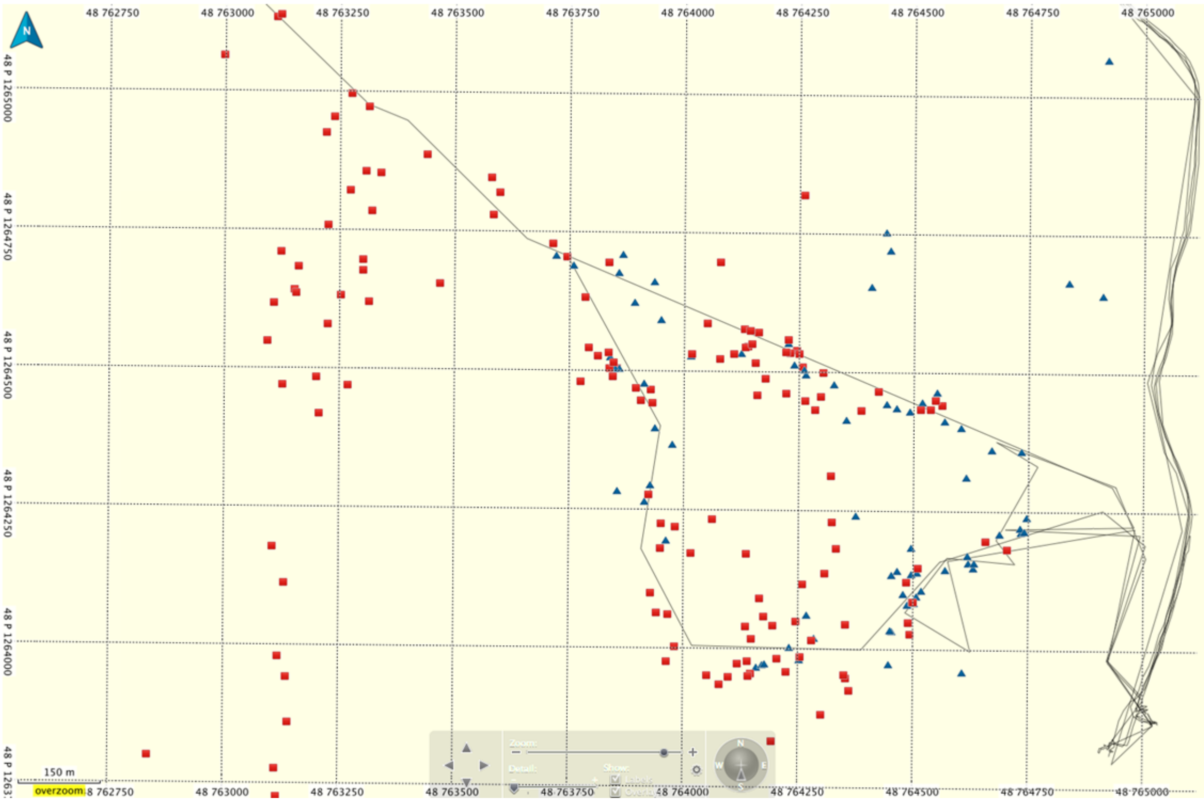

Figure 5.6. Locations of all doucs: dry season and wet season combined. (Red squares are dry season. Blue triangles are wet season.)

These areas were walked during the dry season and the rainy season at a similar frequency; the pattern above reflects that the animals were not present in this western area during the rainy season.

5.4 Locations of feeding trees

Figure 5.7 illustrates the locations of observed feeding trees. This study sampled many of the trees fed upon, but was not able to sample all of the trees observed. Even with this caveat, a good sample of leaves was chemically tested for nutritional composition (see chapter six). Doucs feed on trees that had an average DBH of 45.51 cm, a maximum of 129 cm, and a minimum of 9 cm.

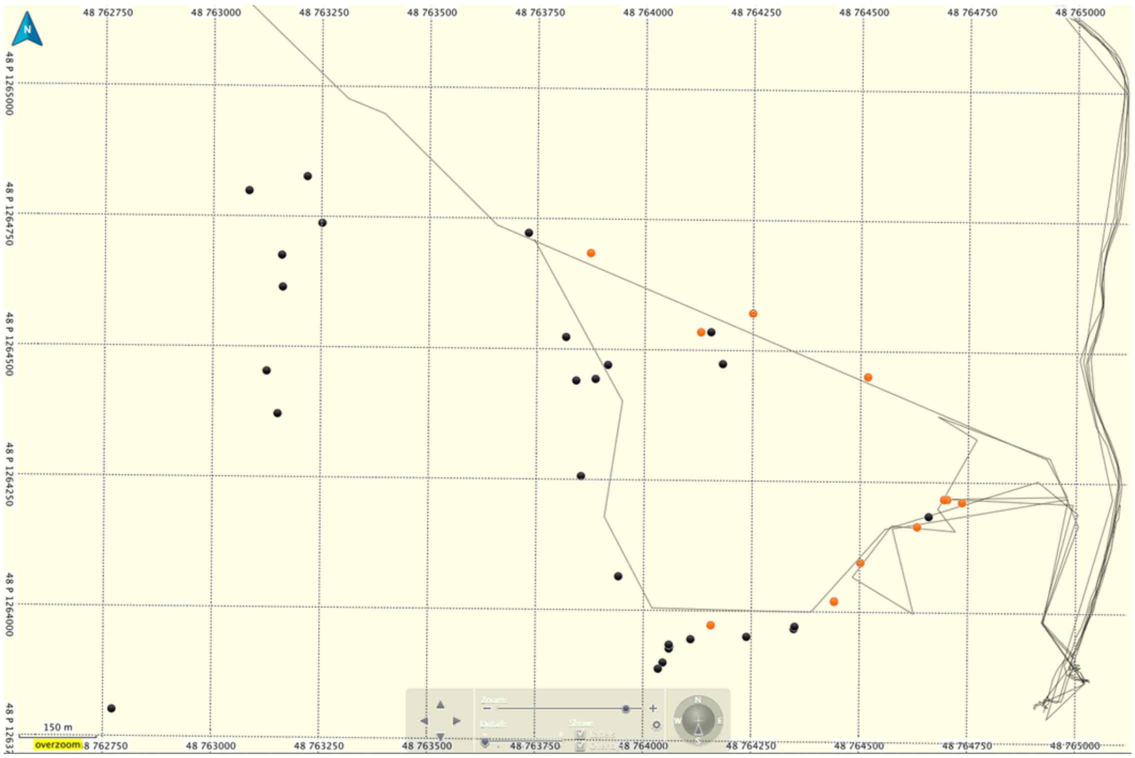

Figure 5.7. Locations of observed feeding trees. (Dry season are black circles. Rainy season are orange circles.)

Figure 5.8 overlays the doucs encountered during the rainy season with the locations of trees feed upon. Many of the trees in this area are deciduous trees and would be in flush with new growth during this time, which may explain why the doucs are also more active in the same area during the rainy season.

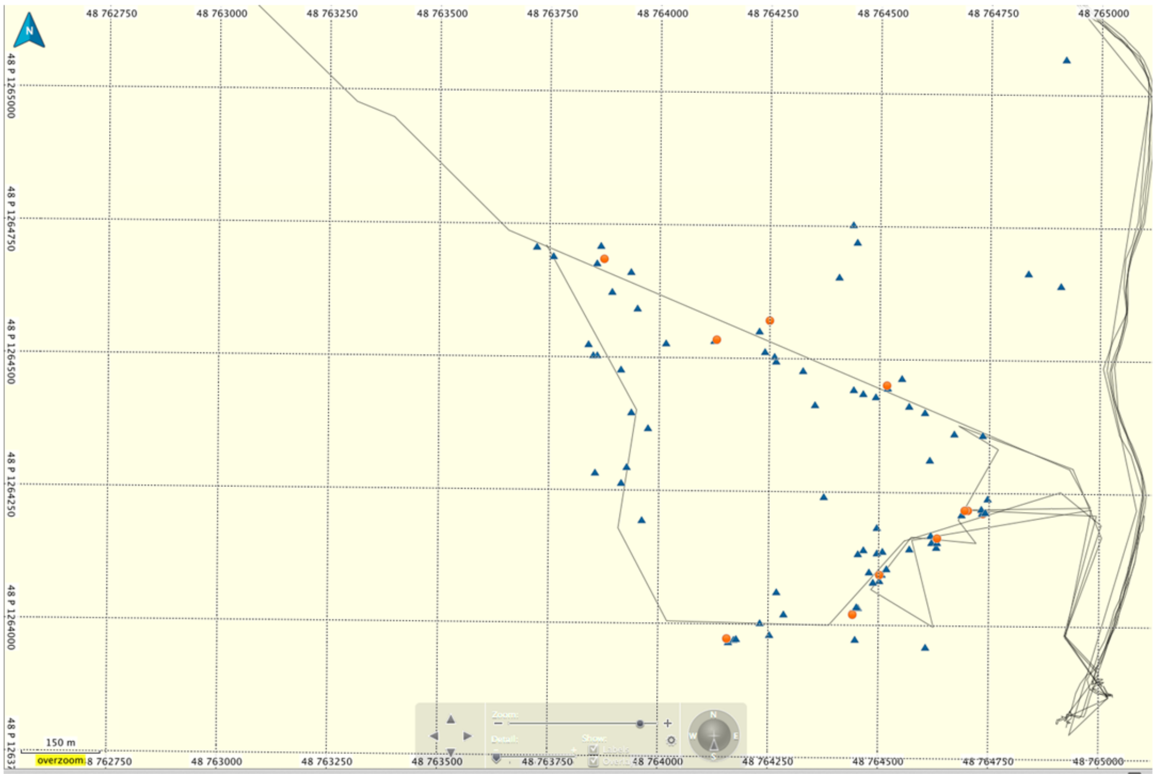

Figure 5.8. Feeding trees and douc encounters in the wet season. (Blue triangles are douc encounters. Orange circles are wet season feeding trees.)

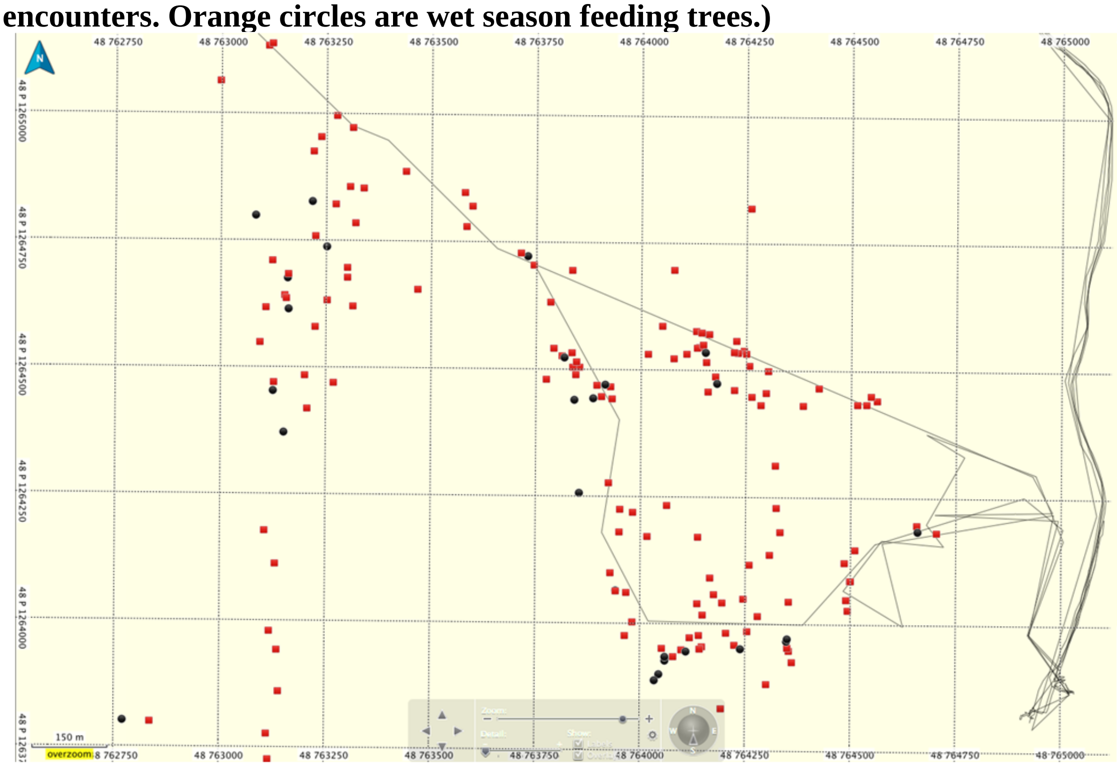

Figure 5.9. Feeding trees and douc encounters in the dry season. (Red squares are dry season douc encounters. Black circles are dry season feeding trees.)

Figure 5.9 shows the douc encounters and trees fed upon during the dry season. This map shows a greater emphasis on areas to the west. Figure 5.10 combines both seasons for douc encounters and trees fed upon. It illustrates a seasonal shift in habitat use for the douc groups.

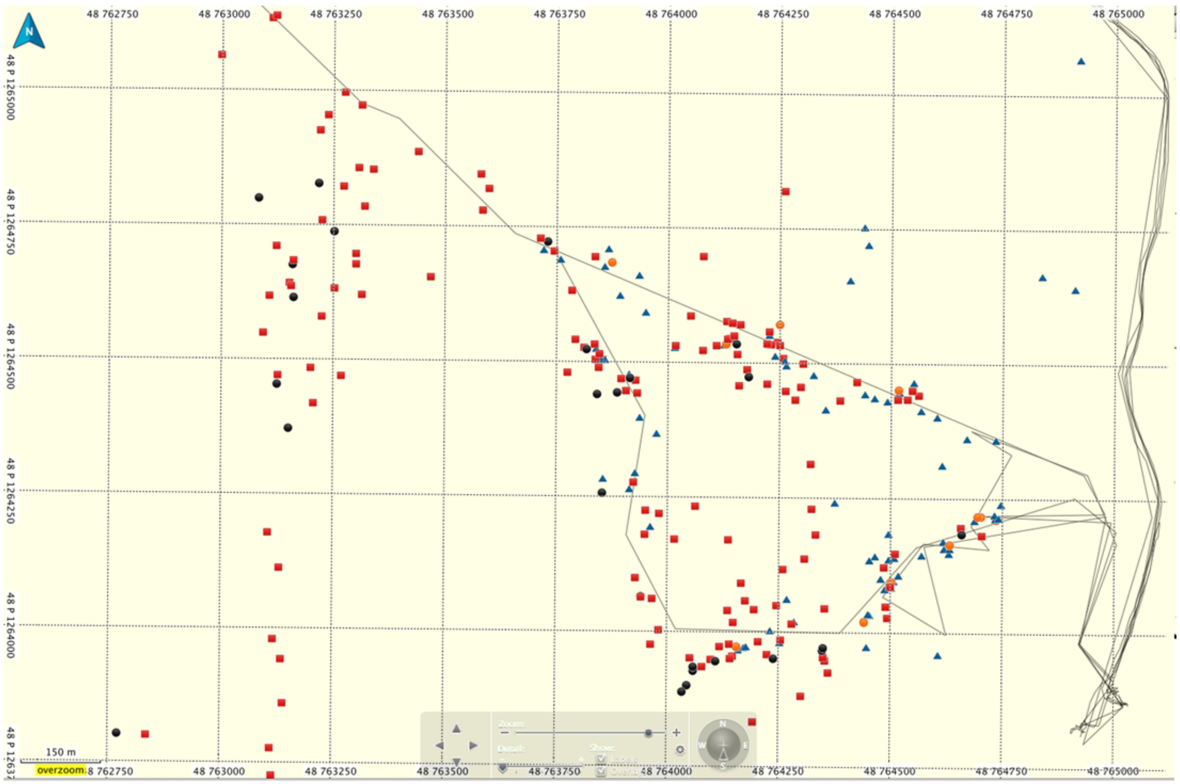

Figure 5.10. Feeding trees and douc encounters seasons combined. (Red squares are dry season douc encounters. Blue triangles are wet season douc encounters. Black circles are dry season trees. Orange circles are wet season trees.)

5.5 Discussion

The ranging behavior of black shanked-doucs is still poorly understood. This study has the same limitation that Rawson (2009) noted in that individuals were not recognized and full daily follows were not well represented in either sample. This research does demonstrate, though, that the doucs vary in their habitat use from the wet season to the dry season. In particular, the doucs travel further west during the dry season, likely tracking *Afzelia* trees for feeding resources. *Afzelia* trees are present throughout the entire study area in lower densities, but the area to the west of the headquarters' area has a much greater density of these trees in one location. This result corresponds with observations made by Rawson (2007) regarding the consumption of *Afzelia*, a preferred and actively selected resource, found in the Seima Biodiversity Conservation Area. Range flexibility is noted as a response to seasonal pressures and changing food availability that allow an animal to temporally shift habitats (Hemingway and Bynum, 2005); this habitat shifting allows the animals to move across the landscape during the seasonal shifts.

A basic principle of conservation management is an understanding of the habitat preferences of a species (Butterfield et al., 1994). Doucs prefer evergreen forests, but are also noted to be behaviorally flexible: varying in the habitats they live and foods they ingest (Lippold, 1998). This study found the doucs living in evergreen, semi-evergreen, and mixed bamboo/ broadleaf forests. These results can be directly compared to Rawson's (2009) habitat description of four habitat types the doucs lived in: evergreen, semi-evergreen, mixed deciduous, dry deciduous dipterocarp forests; as well as Hoang Minh Duc's (2007) habitat data for Nui Chua NP: dry thorny scrub and woodland, dry deciduous forest, sclerophyll forest, and submontane evergreen forest. And finally, in Phouc Binh NP the habitats sampled were evergreen forest, mixed bamboo/ broadleaf forest, semi-deciduous forest, and mixed coniferous/ broadleaf forest. Based on these data, doucs can occupy a wide variety of habitats, but have a common element of presence in evergreen and semi-evergreen areas. I argue that this flexibility underlies the doucs ability to move from one habitat structure to another as the seasons change. These results concur with Hoang Minh Duc (2007) who also argued that doucs were shifting habitats in Nui Chua NP during the dry season to move to areas that had an abundance and diversity of evergreen plants to feed on. In Cat Tien NP, this westward shift of doucs during the dry season moved them into an area with a greater concentration of *Afzelia* and other evergreen trees and out of the more deciduous *Lagerstroemia* dominated forests. Chapter 4 detailed the forest structure of each transect, and specifically, the Ben Cu transect is located in the eastern portion of the headquarters' area. It was described as a *Lagerstroemia* dominated area, but it also contained a mix of deciduous and evergreen trees. As phenology transects showed, during the dry season, the trees along the Ben Cu transect and in the surrounding forest lost up to fifty percent of their leaves. These results lend greater support for the doucs migrating out of this eastern area toward the more *Afzelia* dominated areas to the west.

The social grouping of the doucs varied through this study. Previous studies of the doucs have described variation in social organization ranging from single males, one male units, multi-male multi-female units, and multi-tiered organizations (Lippold, 1998; Hoang Minh Duc, 2007; Rawson, 2009). The doucs in Cat Tien NP seem to exhibit a fission-fusion social organization with smaller groups of one-male units banding together to form much larger groups (40+ individuals). The largest grouping of doucs was observed during the dry season (February, 2010), this is counter to observations by both Hoang Minh Duc (2007) and Rawson (2009) that larger group sizes were found during the rainy season. This has been argued to be reflective of greater amounts of resources available allowing larger aggregations of animals (Hoang Minh Duc, 2007; Rawson, 2009). This is likely also a consideration in Cat Tien NP, but further study of more habituated groups would be necessary to better understand these social dynamics.

5.7 Summary

This chapter showed the locations of both black-shanked doucs and their feeding trees in Cat Tien NP. The variation in locations is indicative of seasonal movement through the forest in order to track leaf resources. A clear pattern of western movement by groups is presented as well as patterns of feeding trees shifting. This movement likely reflects tracking *Afzelia* trees where the doucs were seen feeding regularly during the dry season. Chapter 6 will present nutritional information on the leaves selected and rejected by the doucs.

CHAPTER 6: NUTRITIONAL ANALYSIS OF LEAVES INGESTED AND REJECTED

6.1 Introduction

One goal of this project is to describe the nutritional composition of leaves ingested compared to leaves not ingested by black-shanked doucs (*Pygathrix nigripes*) in Cat Tien National Park, Vietnam. While leaves are a readily available resource in the environment, not all leaves are created equal: they often vary in nutrients, secondary compounds, and other chemical constituents (Janzen, 1978; McNab, 1978; Milton, 1978; Dominy and Lucas, 2001; Chapman and Chapman, 2002; Lambert, 2011) Therefore, this study tests hypotheses regarding how the feeding ecology of the black-shanked douc varies across ecologically diverse habitats; and yields an improved understanding of the habitat requirements for doucs, which is essential to developing detailed conservation plans for both this species and the national park.

6.2 Research aims:

Aim 1: To determine the effects of protein to fiber ratios on food item selection.

<u>Hypothesis 1:</u> If the black-shanked douc are selective feeders, then they will actively select leaves that are higher in protein and lower in fiber.

Aim 2: To determine the effects of plant chemistry on food selection.

<u>Hypothesis 2:</u> Black-shanked doucs will select foods that are lower in overall tannins.

6.3 Results

This project evaluated the chemical composition of leaves eaten and leaves not eaten. The leaves were tested for protein content, crude fiber content, and tannin content. In addition, the following macrominerals and trace elements were measured: Calcium, Potassium, and Sodium, and Iron, Copper, Manganese, and Zinc.

The National Research Council (NRC) published a series of volumes delineating the nutritional requirements of many species of animals including nonhuman primates. Table 6.1 lists the nutrient concentrations recommended by the NRC (2003) for the following nutritional components: Crude protein, NDF, ADF, calcium, potassium, sodium, iron, copper, and manganese. In addition, Table 6.1 lists the mean averages of nutritional compounds found in this study. The comparison of my data with the NRC data highlights more variation in the chemical constitutes of the diet for a non-human primate. The NRC has tried to create a generalized nutritional guide for non-human primates, but many of these differences may be attributed to the species of animals present in the NRC sample (non-colobine); as Yeager et al. (1997) suggested, using a ruminant comparison may yield a more accurate nutritional yield. Even with this suggestion, many of results of this study fall with the recommended guidelines.

Parameter	Concentration	This study
Crude Protein %	15-22	17.42
NDF %	10-30	
ADF %	5-15	
Ca %	0.8	0.39
K %	0.4	0.41
Na %	0.2	0.05
Fe mg/kg^{-1}	100	0.04 %*
Cu mg/kg^{-1}	20	28.15
Mn mg/kg^{-1}	20	313.22
Zn mg/kg^{-1}	100	30.81

Table 6.1. NRC recommended nutritional requirements for non-human primates compared to this study. *The nutritional tests at Nong Lam University reported this as a percentage, not mg/ kg^{-1}.

Table 6.1. is the comparison of eaten and non-eaten leaf materials from Cat Tien NP. The variation between the eaten and non-eaten samples were significant for: protein, Ca, K, Na, Fe, and Cu. Table 6.2 also lists if the percentage of leaves eaten were higher or lower in the nutrient measured (e.g. the protein content was significantly higher in the leaves ingested rather than the leaves rejected).

Chemical Test	**p value (Wilcoxon Non-parametric test)**	**Is % of leaves eaten higher or lower in the nutrient?**
Protein	**p = 0.0002**	Higher
Tannin	p = 0.2308	Lower
Crude Fiber	**p = 0.0349**	Higher
Calcium	**p = 0.0013**	Lower
Potassium	**p < 0.0001**	Higher
Sodium	**p < 0.0001**	Higher
Iron	**p < 0.0001**	Higher
Manganese	p = 0.5326	Lower
Copper	**p = 0.0003**	Higher
Zinc	p = 0.0554	Higher
Protein: Fiber	p = 0.1266	Higher

Table 6.2. Comparison of chemical tests for eaten and non-eaten leaves.

Mineral	Ca	Fe	Mg	Cu	Zn	Na	K
Ca	~						
Fe	**$p < 0.0001$**	~					
Mn	$p=0.6514$	$p = 0.0741$	~				
Cu	**$p < 0.001$**	**$p < 0.0001$**	**$p < 0.0269$**	~			
Zn	**$p < 0.0002$**	$p=0.4776$	$p = 0.0933$	**$p < 0.0236$**	~		
Na	**$p < 0.0001$**	$p=0.5325$	$p = 0.1320$	**$p < 0.0208$**	$p = 0.6055$	~	
K	**$p < 0.0001$**	**$p < 0.003$**	**$p < 0.0160$**	$p = 0.7679$	**$p < 0.0017$**	**$p < 0.0001$**	~

Table 6.3. Comparison of mineral content (Wilcoxon tests)

Following Yeager et al. (1997), Table 6.3. lists comparisons of chemical elements grouped by eaten and non-eaten leaf materials. Most comparisons were significantly different, except comparisons between: Ca:Mn, Fe:Mn, Fe:Zn, Fe:Na, Mn:Zn, Mn:Na, Cu:K, Zn:Na.

#	Family	Species	Protein %	Tanin %	CF %	Ca %	K %	Mn mg/kg	Na %	Cu mg/kg	Fe %	Zn ppm
1	Sp 1a	-	36.89	5.09	15.53	0.2	0.78	24.1	0.05	28.9	0.024	89.9
2	Sp 2a	-	35.85	4.18	20.49	0.3	0.85	28.4	0.04	26.9	0.018	71.8
3	Sp 3	-	21.07	4.37	34.25	0.6	0.32	38.6	0.04	23.7	0.038	32.1
4	Annonaceae	*Alphonsea gaudichaudiana*	11.29	9.46	40.70	0.2	0.30	666.9	0.05	48.6	0.026	19.9
5	Species 2b	-	17.09	5.46	25.68	0.6	0.28	28.6	0.05	9.7	0.023	16.4
6	Species 2c	-	17.41	5.27	35.70	0.7	0.31	32.8	0.06	35.5	0.026	17.5
7	Species 1b	-	18.50	3.64	17.39	0.6	0.37	24.0	0.06	51.5	0.032	25.3
8	Species 1c	-	18.20	4.00	33.78	0.6	0.35	25.5	0.06	17.1	0.026	14.1
9	Annonaceae	*Alphonsea gaudichaudiana*	10.83	8.00	33.52	0.3	0.31	916.9	0.04	35.2	0.029	38.7
10	Annonaceae	*Alphonsea gaudichaudiana*	10.69	7.64	34.74	0.3	0.36	940.5	0.05	25.5	0.036	30.8
11	Annonaceae	*Alphonsea gaudichaudiana*	10.77	8.91	30.85	0.4	0.34	1004.6	0.05	19.2	0.028	19.5
12	Annonaceae	*Alphonsea gaudichaudiana*	10.52	7.64	17.34	0.4	0.27	1012.1	0.04	24.4	0.045	27.2
13	Fabaceae	*Afzelia xylocarpa*	15.92	2.18	34.12	0.1	0.43	5.5	0.04	9.7	0.015	60.2
14	Fabaceae	*Afzelia xylocarpa*	16.18	1.46	43.52	0.2	0.43	5.1	0.04	16.8	0.025	16.1
15	Fabaceae	*Afzelia xylocarpa*	16.99	1.46	33.28	0.1	0.51	5.4	0.05	11.1	0.036	18.9
16	Tetramelaceae	*Tetrameles nudiflora*	17.82	9.82	21.95	0.6	0.44	738.2	0.07	8.4	0.128	35.8
17	Species 3	-	16.23	9.46	35.17	0.5	0.31	29.7	0.06	11.3	0.063	11.5
18	Species 4	-	9.86	8.37	26.99	0.2	0.38	397.6	0.05	47.5	0.062	19.9
19	Species 5	mimosoid	18.77	10.19	-	-	-	-	-	-	-	-
20	Fabaceae	*Afzelia xylocarpa*	17.50	3.27	24.09	0.4	0.46	26.7	0.05	83.8	0.022	19.9

Table 6.4 Analytical results from Nong Lam University on nutritional composition of leaves eaten. Unknown species are designated by Species (#), if the trees are the same species then a # and letter are assigned. CF= crude fiber.

#	Family	Species	Protein %	Tanin %	CF %	Ca %	K %	Mn mg/kg	Na %	Cu mg/kg	Fe %	Zn mg/kg
1	Melastomaceae	*Memecylon fruticosum*	6.30	4.37	20.49	1.25	0.07	26.81	0.02	KPH	0.01	6.01
2	Annonaceae	*Polyalthia cerasoides*	9.04	6.55	31.57	2.76	0.08	213.9	0.03	7.4	0.02	16.79
3	Sapindaceae	*Nephelium hypolcuncum*	10.73	8.25	28.47	0.46	0.13	18.81	0.03	6.08	0.02	16.35
4	Papilionoideae	*Dalbergia bariaensis*	8.96	7.28	28.81	2.67	0.08	15.83	0.04	11.02	0.01	7.93
5	Euphorbiaceae	*Cleistanthus myrianthus*	8.75	9.22	28.79	0.36	0.11	899.7	0.03	3.53	0.01	23.7
6	Lythracene	*Lagerstromeia calyculata*	9.20	23.77	15.63	1.34	0.12	211.8	0.03	10.32	0.01	27.34
7	Moraceae	*Streblus taxoides*	12.75	4.37	16.24	1.25	0.1	2505	0.04	3.12	0.02	35.9
8	Bignoniaceae	*Markhamia stipulata*	13.58	4.61	18.04	1.30	0.12	221.6	0.03	11.73	0.03	27.4
9	Dilleniaceae	*Dillenia scabrella*	13.02	3.64	32.32	0.43	0.19	33.67	0.03	KPH	0.02	6.65
10	Sapindaceae	*Mischocarpus sondairus*	8.19	10.19	17.21	0.44	0.1	448.9	0.03	9.09	0.02	10.39
11	Lythracene	*Lagerstromeia sp.*	7.50	22.07	21.78	0.91	0.12	48.91	0.02	5.12	0.01	17.15

Table 6.5 Analytical results from Nong Lam University on nutritional composition of leaves not eaten. (KPH = not detected, detection limit: Cu : 3 mg/kg). CF = crude fiber.

This study can be further compared with a different population of doucs living to the east of Cat Tien NP on the coast of Vietnam (Hoang Minh Duc et al., 2011). Table 6.6 lists the means and standard deviations for leaf materials eaten and not-eaten for both this study and Dr. Duc's study. A difference that immediately stands out in the data sets is protein content: this research showed significant differences in the protein content of eaten compared to non-eaten leaves, while Hoang Minh Duc et al. (2011) reported no significant differences. Further, the doucs in Cat Tien showed an opposite trend from Dr. Duc's study in fiber content; the leaves eaten in Cat Tien were higher in fiber than the leaves not eaten in Cat Tien and higher than both categories in Dr. Duc's results.

Parameter	HMD Eaten Mean (sd) n = 15	HMD not-eaten Mean (sd) n = 6	O'Brien eaten Mean (sd) n = 20	O'Brien not-eaten Mean (sd) n = 11
Protein %	12.3 (3.66)	15.36 (5.17)	17.42 (7.31)	9.82 (2.39)
Fiber %	23.87 (7.26)	16.89 (1.61)	29.43 (8.14)	23.58 (6.48)
Tannin %	6.57 (4.86)	7.61 (6.53)	5.99 (2.9)	9.48 (6.99)
Calcium %	0.60 (0.34)	0.86 (0.94)	0.39 (0.19)	1.20 (0.84)
Potassium %	0.61 (0.25)	0.59 (0.22)	0.41 (0.16)	0.11 (0.03)
Manganese mg/kg	145.25 (131.69)	133.51 (73.24)	313.22 (411.65)	422.32 (739.72)
Zinc mg/kg	23.18 (21.97)	29.29 (7.44)	30.81 (21.15)	17.78 (9.82)

Table 6.6. Comparisons of eaten and non-eaten leaf material between Hoang Minh Duc and O'Brien's studies.

A further analysis between the two studies shows that there are significant differences between the eaten and non-eaten leaf material for protein, crude fiber, and potassium (Table 6.7).

This comparison of data for separate populations of doucs allows for a greater understanding of the dietary breadth for the species.

Parameter	Eaten Leaf material	Non-eaten leaf material
Protein %	**$p < 0.0164$**	**$p < 0.0036$**
Tannin %	$p = 0.8414$	$p = 0.3146$
Crude Fiber %	**$p < 0.0461$**	**$p < 0.0348$**
Ca %	$p = 0.0634$	$p = 0.1445$
K %	**$p < 0.0137$**	**$p < 0.0009$**
Mn mg/ kg	$p = 0.8487$	$p = 0.6153$
Zn mg / kg	$p = 0.114$	**$p < 0.0270$**

Table 6.7. Comparisons of eaten and non-eaten leaf material by element.

6.4 Discussion

Lambert (2011) noted that studying an animal's feeding behavior is more than a matter of academic concern; it is also a priority of conservation work. By better understanding what an animal is actively selecting for while feeding, it is possible to create plans for animals in captivity as well as for national park conservation planning.

This study has collected data that is compared to both the NRC ideal primate nutritional requirements and to the diet of separate populations of doucs also living in Vietnam. These comparisons reveal perhaps useful information about protein needs and selection behaviors: in this study, the mean protein levels of leaves eaten (17.419% crude protein) was within the normal parameters (15-22%) suggested by the NRC (2003) and there was a significant difference in selection for leaves with higher protein (Wilcoxon $p=0.0002$). In comparison, the protein levels of eaten leaves in Hoang Minh Duc et al. (2011) were lower—12.30%-- which is less protein than suggested by the NRC. These studies combined can give a good indication of the variability of this species across their habitats in Vietnam. The higher protein levels found in this study may be due to the parasite loads the doucs in Cat Tien NP are known to have (chapter seven); future work with populations of doucs in Nui Chua NP and Binh Phuoc NP to assess their potential parasite loads could better explain one source of this dietary variation.

Crude fiber is typically argued to be lower in selected food items (Milton, 1979; Oates et al., 1980; Yeager et al., 1997), but it does not appear to be a crucial factor in leaf selection for black-shanked doucs in Cat Tien NP. There was a significant difference in the amount of crude fiber present in leaves eaten compared to not eaten leaves (Wilcoxon p=0.0349). The mean crude fiber ingested was higher in eaten leaves (29.43%) than not eaten leaves (23.57%). Wright et al. (2008) argued that this non-selection for lower fiber content could be mitigated by the gut function and morphology within doucs. Further, though we currently know little about gut retention times for doucs or their overall efficiency for digestion, Caton (1998) described aspects of the *Pygathrix nemaeus* gut morphology that may allow it to break down more of the ingested materials than other colobines.

The doucs ate leaves with lower levels of calcium. While calcium is an important macromineral for the health and nutrition of an animal, the doucs did not select leaves that were higher in calcium. Also, the mean calcium for leaves eaten are below the NRC's (2003) recommendations for nutrient requirements, which raises questions about the genus *Pygathrix* and the use of geophagy to supplement nutritional requirements (Rawson and Bach, 2011).

The trace mineral iron, an essential portion of diet for normal bodily function, is well represented with the selected leaves of these animals. While mean levels for iron are low compared to the NRC (2003) data, the iron levels are significantly higher in leaves selected as food items (Wilcoxon $p < 0.0001$). Further, considerable variation exists between sites: compared to Hoang Minh Duc et al. (2011), leaves eaten had a higher mean iron content of 11.04g/ kg compared to 0.4 g/ kg for leaves eaten in Cat Tien NP. While iron requirements for non-human primates are still not well understood (NRC, 2003), iron absorption has been linked to levels of ascorbic acids (NRC, 2003); this study did not address vitamins and their contribution to the nutritional ecology of the animals.

The flexibility of colobine digestive systems to handle a variety of chemical compounds in varying concentrations may be what allows them to live in these varied habitats (Kay and Davies, 1994). These data presented show differences in the leaves ingested compared to leaves not ingested by the doucs. In addition, the doucs appear to be somewhat selective in the leaves that they do ingest favoring younger leaves over mature leaves, leaves that have higher protein content than lower content, and not having lower fiber content driving selection. The doucs in Cat Tien NP are tracking resources by moving through different habitats and showing seasonal variation in where they are feeding. This flexibility of movement coupled with being able to ingest a wide range of nutritional levels across the taxa may explain how they successfully live in such varied habitats.

6.5 Summary

This chapter reviewed the nutritional elements found in the diet of black-shanked doucs. Aim 1 was partially supported. The doucs did selectively feed on leaves higher in protein, but also fed on leaves that were higher in fiber. Aim 2 was not supported. There was no difference in leaves eaten and leaves not eaten for tannin content. There were significant differences in macromineral content of leaves eaten compared to leaves not eaten for calcium, potassium, and sodium, with higher levels of potassium and sodium, and lower levels of calcium in ingested leaves. There were significant differences in trace mineral content of leaves eaten compared to leaves not eaten for iron and copper. Both of these trace minerals had higher concentrations in the selected foods.

CHAPTER 7: PARASITE ECOLOGY OF THE BLACK-SHANKED DOUC IN CAT TIEN NP

7.1 Introduction

This chapter provides the results of the analysis of the gastrointestinal parasites harbored by a population of black-shanked doucs. Understanding what parasites a given taxon hosts allows for an investigation of their relationships. Huffman (2007) noted that a primate's life is closely linked with the parasites that it carries. Moreover, an animal may be infested with parasites for a number of reasons, but this often does not provide any health risk to the host (Ancrenaz et al., 2003). Finally, Lozano (1991) noted that parasite infection could alter foraging behaviors of animals and listed three influences to diet choice: avoidance of certain food items, ingesting certain foods to change gastrointestinal composition, and self-medication to kill established parasites. Thus, the results in this chapter allow discussion of the relationship between the nutritional components of the diet to the parasitic burden this population carries.

7.2 Research Aim

Aim 1: Investigate parasite presence / absence in black-shanked douc.

While previous parasite research in Cat Tien NP on golden-cheeked gibbons included some information on black-shanked doucs, there was a very small sample size and a lack of a common primate parasite species: *Strongyloides* sp. (Kenyon, 2007). The research presented here focuses on the doucs to investigate if the samples described by Kenyon are representative of the animals in Cat Tien NP. In addition to reporting on presence / absence of parasite species, I also present quantification of infection rates and multiple species infections.

7.3 Statistics used in this section

I consulted with Dr. Paul Sandburg on appropriate statistical tests to utilize for comparing the parasite infection rates. Descriptive statistics were used to initially characterize the sample before inferential statistics were used to compare multiple samples. In order to analyze the parasite presence and abundance, chi-square and Fisher's Exact test were used. The chi-square test is a 2 x 2 contingency table used to show likely parasite infection. The data were compressed into absence or presence of parasite, and intensity of infection was ignored. The Fisher's Exact tests were run to compare this sample with Kenyon (2007); tests were run at α of 0.05.

7.4 Results

7.4.1 Presence of Parasites in the sample

Forty eight black-shanked douc fecal samples were collected in Cat Tien NP during May 2012. Forty of the 48 samples (83%) included at least one parasite. There were a total of 552 parasites observed within the samples and included a variety of Nematodes--*Strongyloides*, *Trichuris*, *Physaloptera*, *Enterobius* (pinworms); Cestoda (tapeworms); mites; louse; and 2 unidentified parasites. Black-shanked doucs were more likely to be infected by gastrointestinal parasites than to be free of infection ($p<.001$, $\chi^2=21.333$, $df = 1$). Fisher's exact test showed that doucs analyzed by Kenyon (2007) are statistically no different and have the similar parasite presence and absence likelihood as this current sample, but with different parasites present (one-tailed p = 0.18, two- tailed p = 0.33). One important point to note is the small sample size that was analyzed by Kenyon (n= 8) compared to the present sample (n= 48). Interesting results in this sample include the lack of protistan parasites, a parasite common in Kenyon's sample and the abundance of *Strongyloides* sp. (present in 33%), a parasite that Kenyon did not record (Table 7.1). The only species infected by *Strongyloides* sp. in Kenyon's study were the gibbons (2.1%, n = 47) and stump-tailed macaque (33.3%, n = 3).

Host Species Number of independent samples	Pig 1	Mouse Deer 1	Stump-Tail 3	Long-Tail 2	Pig-Tail 4	Gibbon 47	Douc 8	This Study 48
NEMATODE								
Trichuris sp.			66.7	50	25	59.6	37.5	23
Physaloptera tumefaciens				50		14.9	12.5	2.1
Hymenolepsis nana/ ascaris ova						2.1		
Rhabditis						4.3		
Trichostrongylus type						2.1		
Strongyloides			33.3			2.1		33
Hookworm	*				25			
Oseophogostomum	*							
Enterobius sp.								10
Spirometra	*							
PROTOZOA								
Entamoeba histolytica / dispar	*		66.7		25	12.8	37.5	
Entamoeba chattani						12.8	12.5	
Iodamoeba buetschlii	*		100		25	10.6	25	
Entamoeba hartmanni	*		33			6.4	12.5	
Entamoeba coli			33			8.5	12.5	
Endolimax nana						6.4	12.5	
Ballantidium coli			33	50		17	12.5	
Blastocystis hominis						6.4		
Trichomonas			33	50	25	21.3	12.5	
Chillomastix mesnili						2.1		
OTHER						14.9		
Unidentified larvae 1						2.1		
Unidentified larvae 2					25			
Unidentified larvae 3								
Unidentified larvae 4								2.1
Unidentified larvae 5								2.1
Unidentified Mite								2.1
Unidentified Louse								2.1
Unidentified cestoda								52

Table 7.1. Parasite species prevalence from selected mammals within CTNP (* = parasite) adapted from Kenyon (2007). The numbers in the table are the percentage of fecal samples harboring the specific parasites.

7.4.2 Parasites harbored in Black-shanked douc

The parasites infecting the doucs are listed below in the order of prevalence. Figure 7.1 lists the total percentages of parasites found in the total sample.

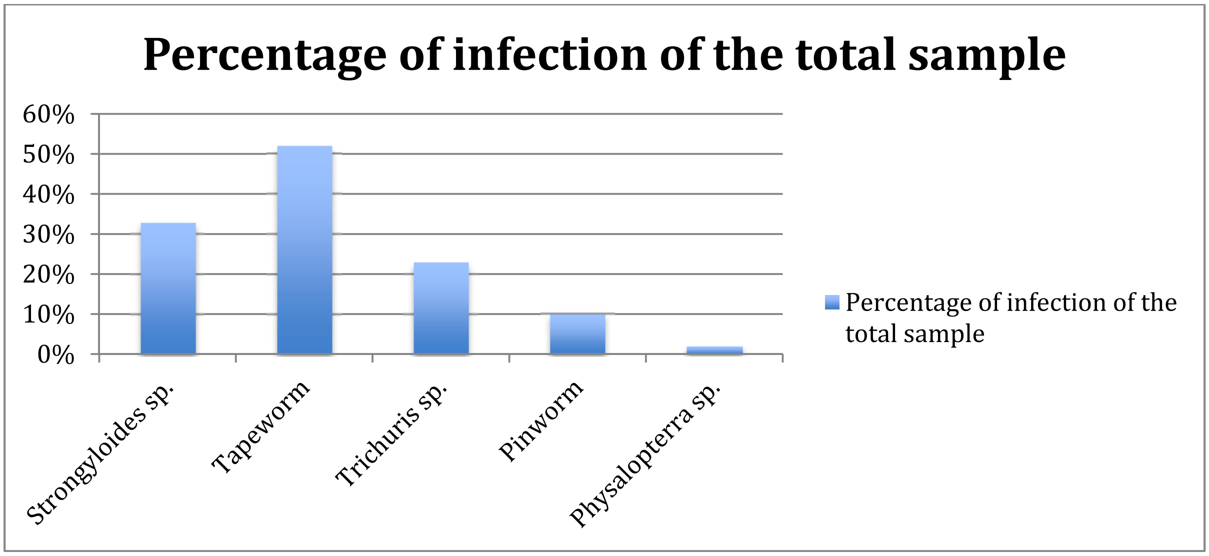

Figure 7.1. Percentages of infection by species for the total sample.

7.4.2.1 *Strongyloides*

Strongyloides sp. was the most abundant parasite with 275 eggs observed in 16 of the 48 specimens (33%) (Figure 7.2). The direct smear method found 25 eggs while the float method found 250 eggs.

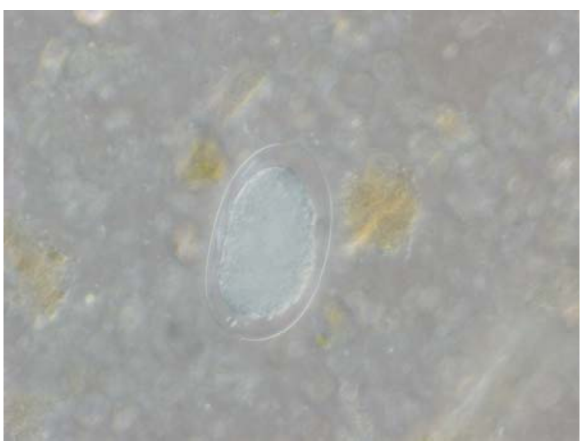

Figure 7.2. *Strongyloides* from Sample #9 viewed at 40x.

7.4.2.2 Tapeworm

Tapeworms were the second most abundant parasite with 257 eggs observed in 25 of the 48 specimens (52%) (Figure 7.3). The direct smear method found 255 eggs and the float found two eggs.

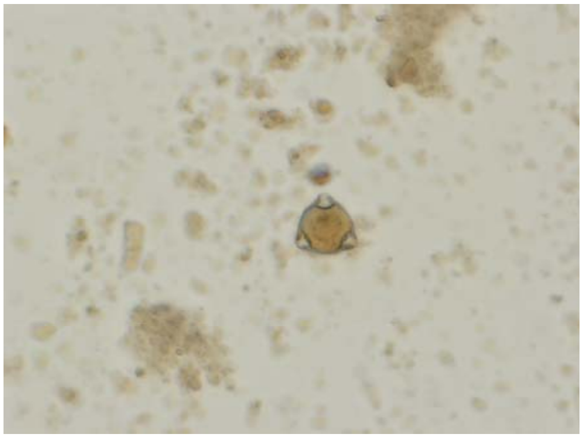

Figure 7.3. Tapeworm sample #37 viewed at 40x.

7.4.2.3 *Trichuris*

Trichuris sp. was the third most abundant parasite found in 11 of the 48 specimens (23%). There were 20 eggs observed; 17 found in the direct smear method and 3 found in the float method (Figure 7.4).

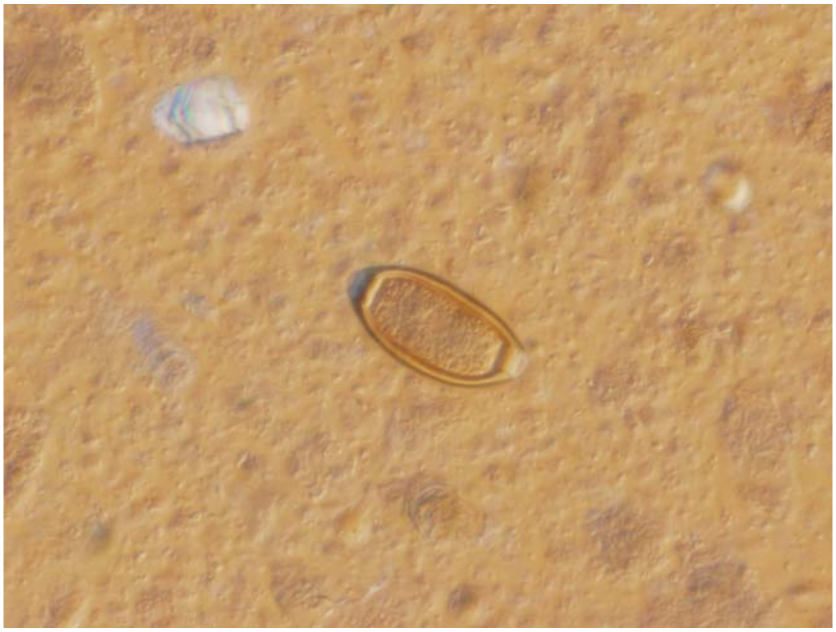

Figure 7.4 *Trichuris sp.* sample #4 viewed at 40x.

7.4.2.4 Pinworm

Pinworms were found in 5 specimens (10%). Both eggs (Figure 7.5) and a mature worm (Figure 7.6) were observed.

Figure 7.5. Pinworm from Sample #46 viewed at 40x.

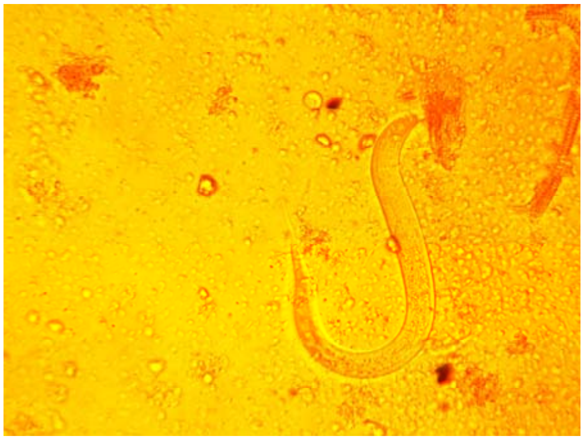

Figure 7.6. Adult pinworm from Sample #1 viewed at 40x.

7.4.2.5 Nematodes: *Physalopterra*

Physalopterra were found in one specimen (Figure 7.7).

Figure 7.7. Nematode sample #40 viewed at 40x.

7.4.3 Concurrent Infections

There were a number of concurrent infections present with 28 (58%) of the specimens having more than one parasite present. I categorized the samples by the number of infections (Figure 7.8) and the parasites present within the samples (Appendix 6).

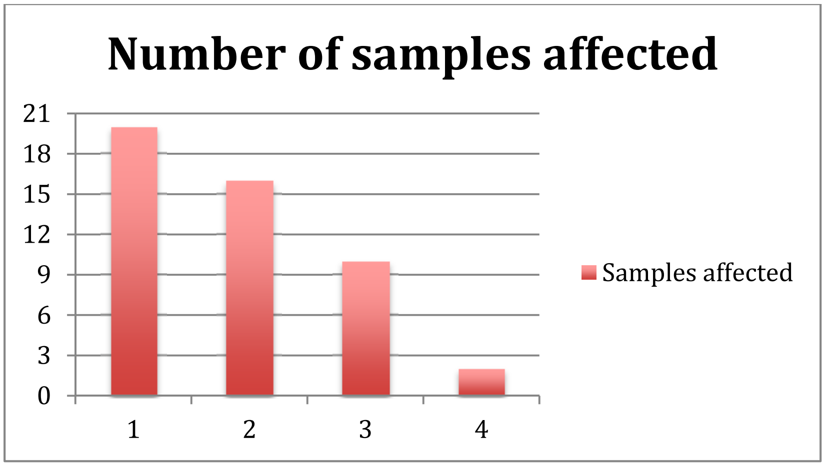

Figure 7.8. Number of parasites present in each sample.

7.5 Discussion

In this study, 83% of all samples contained parasites. Black-shanked doucs were more likely to be infected by gastrointestinal parasites than to be free of infection (p<.001, χ^2=21.333, *df* = 1). While I expected to find parasites based on Kenyon's study, this study found much higher rates of infection from both *Stongyloides sp.* and *Enterobius sp*., which were not present in her previous sample. The presence of these species can be detrimental to the health of the organism; effects of each parasitic organism can vary from being benign to causing bouts of diarrhea or death in more severe cases. Some parasite species are more dangerous to their host than other species. The virulence of the parasite species can be the important factor: a more virulent parasite can cause greater damage to the host and can lead to death, while a less virulent parasite may give the host no negative impacts to the host (Nunn and Altizer, 2006). Examples of parasitic organisms leading to death can vary by host species, but included ticks on *Papio*, tapeworm larva on *Theropithecus*, and bot flies in *Alouatta*. Parasitism can also be a driving factor in the nutritional status of animals (Huffman, 2011).

7.5.1 Diet

As we saw in Chapter 6, this study found that doucs select leaves that are higher in protein, which is an important nutrient for both the building and repair of body systems. Interestingly, protein intake appears to be important for physiological and immunological parasite defense (Lozano, 1998) and as Van Houtert and Sykes (1996) discussed, increases in protein ingestion in ruminants may be a method of mitigating parasite loads. For example, the host's resistance to nematodes establishing themselves in the GI tract or to developing further infection can be affected by the amount of metabolisable protein in the diet. Additional experimental work with sheep found that increases in protein in the diet enhanced the resilience of the host to nematode infections (Van Houtert et al., 1995). Though the current study did not identify individuals to give health and parasite status, or nutritional status, future work on a habituated group with identified individuals could confirm a direct link between protein levels in dietary food items and parasitism at the individual level within non-human primates.

7.5.2 Combating parasites

Primates have been recorded engaging in self-medication through geophagy (Knezevich, 1998; Krishnamani and Mahaney, 2000; Hoang Minh Duc and Baxter, 2006; Huffman, 2011; Rawson and Luu Truong Bach, 2011; Pebsworth et al., 2012) and eating medicinal plants (Huffman, 2011); these behaviors seem to allow the animals to either acquire minerals not within their diets or to ease gastrointestinal distress/ parasite infection. *Macaca mulatta* were observed ingesting large amounts of kaolinitic clays (i.e. Kaopectate®) to counteract the effects of parasite infections (Knezevich, 1998). This strategy may help explain in part why the doucs in Cat Tien NP were seen coming to ground in a dry riverbed, a behavior which has been recorded in other populations of doucs in both Vietnam (Hoang Minh Duc and Baxter, 2006) and Cambodia

(Pollard et al., 2007; Rawson and Luu Truong Bach, 2011). Though the reasons for terrestrial behavior in this study are unclear, the fact that the animals often carry large, parasite burdens suggests that they may be ingesting soils in an effort to ease infection distress.

7.5.3 Seasonality

Additional study on the parasite loads of black-shanked doucs would be greatly enhanced by a consideration of seasonal changes. Huffman et al. (1997) analyzed seasonal trends in infection rates in chimpanzees across the wet and dry seasons. They reported significant differences in infection rates during the rainy season for *Oesophagostomun stephanostomum*, but no differences in *Trichuris trichiura* and *Strongyloides fuelleborni*. While my research does not take seasonality into account for infection rates, this is a potentially promising direction for future work.

7.6 Summary

This chapter provides an introduction to the parasite ecology of black-shanked douc in Cat Tien NP. The samples were significantly more likely to be affected than unaffected. While carrying a parasite load does not necessarily mean an increased likelihood of death or decreased fecundity, it was interesting to note the presence of a previously unreported species of pathogenic parasite (*Strongyloides* sp.) in this population of doucs.

CHAPTER 8: DISCUSSION, CONCLUSIONS, AND FUTURE DIRECTIONS

8.1 Introduction

This dissertation has examined the ecology of *Pygathrix nigripes*, the black-shanked douc, focusing on forest structure, ranging behavior, nutritional ecology, and parasite ecology in Cat Tien National Park, Vietnam. This narrative provides the highlights of this research and addresses the interconnections of the results to provide a broad picture of these animals. Finally, future research topics are addressed.

8.2 Discussion

This dissertation focused on the ecology and conservation of the black-shanked doucs in Cat Tien NP. In order to be better informed on these topics this study looked at forest structure, ranging behavior, feeding and nutritional ecology, and the parasites harbored by these doucs. All of these lines of evidence are connected in understanding how the doucs are utilizing the habitats located in Cat Tien NP. These data are also compelling when compared to the works of Hoang Minh Duc and Ben Rawson that occurred in protected areas with substantially different habitats.

The forests of Cat Tien NP are not a homogenous environment: the forest consists of evergreen, semi-evergreen, deciduous, semi-deciduous, bamboo dominated, riverine and galley forest, seasonally flooded areas, habitually drier grasslands, and mixes of all possible combination. In order to understand that there is any selection for habitat going on, we first need to show that the environment is not homogenous. In order to do this three transects were established in areas where doucs were routinely seen. These areas were described in chapter 4: Bau Sau (mixed evergreen and deciduous forest with the smallest trees described along the transects), Da Co (mixed evergreen and semi-deciduous with the largest trees recorded on the transects), and Ben Cu (primarily deciduous semi-evergreen dominated by *Lagerstromia* sp.

trees). Each of these transects was measured and described in order to understand the timing and availability of resources: leaf material, fruits, and flowers. This information is directly important when looking at feeding selectivity for the doucs. Doucs preferentially select new leaves over mature leaves, but they have been observed to eat mature leaves on occasion (Hoang Minh Duc, 2007). This study did not record doucs eating mature leaves, but when trying to understand the dietary ability of the animals it is useful to be able to not rule out certain behaviors. Doucs in Cat Tien NP preferred habitats that were more evergreen in nature, moving out of areas that more deciduous and lost their leaves during the dry season. The understanding of the seasonal variation of the transects allows for a greater interpretation across the rest of the landscape. Once these transects have been described then you can add animal occurrences over top of these areas to see how the animals are interacting with the habitat. By walking the forest trails and transects recording GPS data for any douc encounters I am able to show variation in habitat use across the wet and dry seasons. I focus on the headquarter area and the Ben Cu transect for this discussion. When evaluating where the animals were concentrating activity between the seasons a map with GPS locations was used to show that the doucs are shifting habitat use in response to the seasonal change- the loss of leaves in certain areas and the tracking of resources to the West of this region. I argue that the seasonal shift of doucs in the headquarter area is in response to following *Afzelia* trees as they bud and produce new leaves early in the dry season. *Afzelia* trees are present throughout the entire area, but are found in greater concentrations to the west of the headquarter area. The GPS data tracks these movements with greater concentration of activity in the South and Southeast of the area during the wet season and then as the forest loses leaves the GPS data shows that the doucs are encountered in the western section of the region where *Afzelia* are more prevalent. While *Afzelia* are not the only tree fed from, they are an important keystone resource for the doucs in Cat Tien NP and the conservation management of this species in the park needs to take action to protect these trees for all of the animals. Nutritionally, looking specifically at *Afzelia* samples, they fall in the average range for protein content of selected leaves (mean = 16.5%, mean for all leaves = 17.42%) and within the average range of crude fiber content (mean = 33.91%, mean for all leaves = 29.43%). This can be interpreted as falling within a normal range for diet of non-human primates and is reasonable for maintaining nutritional requirements during the dry season when less overall leaf material is available in the forest. The comparison of these data to the non-eaten sample also shows clear patterns of differences. The non-eaten leaf material was far lower in crude protein (mean = 9.82%) and also lower in crude fiber (mean = 23.58%). I argue that while protein may be driving selection of leaf material for ingestion, crude fiber does not seem to be a digestive inhibitor for the species. Additional work on the specifics of the douc digestive system would be instructive as to how they are overcoming these higher fiber loads. The final part of the dissertation is the parasite analysis of the doucs. This piece, while mostly a preliminary count of species present and single or multiple infection rates- allows for inference on the interactions of the primate community. The doucs are primarily an arboreal but they do come to the ground on an infrequent basis. This changes the dynamic of parasite infection by allowing them to come into contact with species that spend part of their life cycle in the ground. It is also important to look at what other species of non-human primate the doucs are encountering in these forest habitats. In the headquarter region you routinely encounter pig-tailed macaques and golden-cheeked gibbons. These primates different significantly from one another in habitat usage; the golden-cheeked gibbons stay in the tops of the trees, while the pig-tailed macaques range from the ground to the tops of the trees. Owing to the habitat overlap of these animals, sharing feeding trees, sleeping sites, and locomotor pathways; direct or indirect fecal contact can allow for the spread of numerous parasites across these vertical divides. The doucs and gibbons never need to go to the ground if the parasites are making their way into the canopy with the more terrestrial pig-tailed macaques. This would be an interesting avenue to pursue to check and monitor infection rates and pathways of transmission between the species. Also having direct nutritional and parasite health measures would allow for a better understanding if the parasites are harmful or just another fact of life for these monkeys.

8.3 Conclusions

In Chapter 4, I described the forest habitat of Cat Tien NP based on the three transects that were established to measure: phenology, forest structure, and soil chemical composition. These transects captured the diversity of habitats in Cat Tien NP with variation of tree height, DBH, and basal areas on the trees measured. The three transects selected within Cat Tien NP do demonstrate variability in the habitat that the doucs live in; and these transects do represent a good sample of the forest. Cat Tien NP is a seasonal environment - there are periods of relative abundance and relative dearth of leaves. As discussed in Chapter 5, this seasonal variation is likely causing the doucs to move across the landscape tracking resources. All of the transects experienced the greatest amount of leaf flush starting in March and continuing until June. This reflects the change in seasons and the beginning of the rainy season in Cat Tien NP. Fruit was not available on the transects at all points throughout the year and fruiting levels are different across the range of douc habitats. Feeding on fruit was not a focus of this research; it was observed directly only one time for fruit and one time for seeds. Hoang Minh Duc (2007) reported fruit eating comprising 16.79% of the diet in the dry season and 34.02% for the wet season, while Rawson (2009) reported fruit eating at 9.77% and seeds comprised 39.7% of the diet. This variation in fruit abundance in different forests may be one factor contributing to larger population numbers in other forest areas across the entire range of douc habitats. The final habitat measure addressed was soil chemical composition; while only a preliminary analysis, soil chemistry does change between the seasons specifically in the amount of available nitrogen in the soil. Further investigation into soil chemistry and leaf chemistry would give a better understanding of the seasonal variation and possible stressors to animals relying on the resources.

Building on Chapter 4, Chapter 5 addresses ranging behavior of the doucs through seasonal change of habitat usage. Chapter 4 showed that the forests vary over the seasons; Chapter 5 directly addressed that doucs ranging behavior changes between the dry season and the wet season. The douc groups centered in the headquarters' region were shown to move from east to west from the wet to dry season. This ranging change is likely the outcome of resource tracking through the forest, specifically, following the *Afzelia* as they bud and bloom through the dry season. As discussed in chapter 5, *Afzelia* are present throughout the entire study area in lower concentration, but are found in a greater concentration to the west of the headquarters' region. This seasonal ranging and tracking of habitat type and resource is similar to other doucs in both Nui Chua NP (Hoang Minh Duc, 2007) and Seima Biodiversity Conservation Area (Rawson, 2009).

Chapter 6 focused on the nutritional ecology of the doucs. Chemical analyses were undertaken to better understand leaf selection including the following components: protein content, crude fiber content, tannin content, calcium, potassium, sodium, iron, copper, manganese, and zinc. The doucs are actively selecting leaves that are higher in protein content. Lower levels of fiber content are not driving selection of leaves eaten, as the fiber content was higher in ingested leaves than non-ingested leaves. Tannins were also not a significant driver of leaf selection in this sample. Potassium, iron, sodium, and copper were all significantly different between eaten and non-eaten leaves with higher content present in leaves selected. This nutritional data coupled with Hoang Minh Duc's data give a broader understanding of the nutritional flexibility of these animals. If they had an extremely narrow dietary niche, then we would see little differences between studies. This is not the case and we see the doucs living in wetter and drier habitats, but still meeting their nutritional requirements. This research provides more information on the micronutrient content of foods selected by a colobine; and is comparable to the work of Yeager et al. (1997) for *Nasalis* to demonstrate the variability in micronutrient requirements.

Chapter 7 investigated the parasite ecology of the doucs in Cat Tien NP; doucs had been shown to harbor parasites in previous studies (Kenyon, 2007). The doucs were more likely to be infected with parasites than to not harbor any parasites. Forty-eight black-shanked douc fecal samples were collected in Cat Tien NP during May 2012. Forty of the 48 samples (83%) included at least one parasite. There were a total of 552 parasites observed within the samples and included a variety of Nematodes--*Strongyloides*, *Trichuris*, *Physaloptera*, *Enterobius* (pinworms); Cestoda (tapeworms); mites; louse; and 2 unidentified parasites. Though the current study did not identify individuals to give health and parasite status, or nutritional status, future work on a habituated group with identified individuals could confirm a direct link between protein levels in dietary food items and parasitism at the individual level within non-human primates. If, as Van Houtert et al. (1995) argued, increased protein intake in ruminants can mitigate parasite infection, the fact that these doucs are selecting for higher levels of protein may in part be related to this demonstrated parasitic influence. Future studies on seasonality of infection rates coupled with recognition of individuals would greatly clarify this picture.

Any good research quite often leaves more questions to be answered; this narrative leads toward many additional questions, some of which I discuss below.

8.4 Conservation

A conservation action plan for the species can be formulated for implementation in Cat Tien NP and across their range with information from this dissertation. Two main foci in conservation work are *ex situ* and *in situ* situations. The *ex situ* conservation efforts are useful for rescue centers in Vietnam such as the Dao Tien Primate Rescue Center (Cat Tien NP), Cu Chi Wildlife Rescue Center (Cu Chi), and the Endangered Primate Rescue Center (Cuc Phuong NP). All of these centers occasionally keep black-shanked doucs and the nutritional information presented in this dissertation can help to formulate naturalistic diets for the animals in captivity. Along with the nutritional information, specific plant species that the doucs focus on can be grown to feed the animals. Additionally, the ecological information on habitat preference is useful in assessing future release sites for animal reintroductions. The *in situ* conservation strategies should include: protection of preferred habitat areas, replanting of important tree resources, or application of species specific logging operations in order to keep keystone tree resources available in the forests (Chapman et al., 2003). This last strategy has been suggested as a way to assist in the development and management of endangered species while still accounting for anthropogenic influences within the forests. The combination of *ex situ* and *in situ* conservation strategies would allow for better nutritional status of animals in rescue centers and better access to resources for animals living in forests. This protection would undoubtedly protect not only black-shanked doucs, but also many other species that live sympatrically with the doucs.

8.5 Future directions

8.5.1 Feeding ecology

After completion of this research, it is clear that there are many additional avenues of study to pursue. I want to continue working with the black-shanked douc in Cat Tien NP on feeding ecology and hope to use a mechanical tester to gain comparable data on toughness values for leaves ingested in wild primates to compare with captive data (Wright et al., 2008). In addition to mechanical properties, continued work with the animals may yield better seasonal data to add to this research on nutritional properties of foods ingested. Further comparisons of nutritional data collected on black-shanked doucs at other locations in southern Vietnam will continue to yield a better understanding of dietary requirements, habitat requirements, and behavioral flexibility within this species. Furthermore, we are now at a better place to be able to understand the variation within the genus *Pygathrix* across all of its range.

8.5.2 Parasite ecology

The parasites presented in this work only show presence and absence for one month of the year. It would be informative to return for a second round of collection during a different time of the year to see how season might affect parasite infections within the black-shanked douc. These studies coupled with fecal collection at a number of other sites within southern Vietnam would inform us on parasite load and variation across habitats. In addition, I have analyzed silvered langur (*Trachypithecus germaini*) feces collected by Dr. Covert at Kien Long, Kien Giang province and found a number of parasite species infecting these langurs. A study conducted on three separate populations of silvered langurs in various levels of anthropogenic change and contact would be very informative to health concerns for this species and future translocation implications.

I aspire to continue to work on the ecology and conservation of many primate species in Southeast Asia and continue to foster the relationships that I have built over the past 9 years with Vietnamese, Cambodian, and other international scientists and conservation workers.

Eight years ago while defending my Master's thesis my committee asked me if I wanted to continue working in conservation and if the animals had any chance of survival. Here we are eight years later, and while there are still serious conservation concerns in Vietnam- we have not lost any primate species in Vietnam. We are seeing stronger commitments from the Vietnamese government and local peoples to preserve these amazing creatures. I still consider myself fortunate that I work in such a remarkable place with wonderful colleagues and a wealth of opportunities to continue to help conserve these creatures.

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APPENDICES

Category	Concentrations
High	145 kg km^2/15.24 cm soil
Medium	73 kg km^2/15.24 cm soil
Low	18 kg km^2/15.24 cm soil

Nitrogen content per category.

Category	Concentrations
High	29 kg km^2/15.24 cm soil
Medium	9 kg km^2/15.24 cm soil
Low	3.6 kg km^2/15.24 cm soil

Phosphorous content per category.

Category	Concentrations
High	73 kg km^2/15.24 cm soil
Medium	36 kg km^2/15.24 cm soil
Low	18 kg km^2/15.24 cm soil

Potassium content per category.

Appendix 1. Element concentration by LaMotte soil analysis kit.

Appendix 2. Trees located on the Bau Sau transect. Unidentified trees are labeled as Sp.

Bau Sau Transect			
Sample #	Species: Vietnamese	*Species: Genus species*	Family
1	Dâu da	*Baccaurea ramiflora*	Euphorbiaceae
2	Sp 1		
3	Muong / Na	*Cassia sp.*	Caesalpininoideae
4	Lòng mang lá nhỏ	*Pterospermum grewiaefolium*	Sterculiaceae
5	Sp 2		
6	Sp		
7	Thị (cẩm thị)	*Diospyros maritina*	Ebenaceae
8	Sp 3		
9	Sp 3		
10	Săng mây	*Sageraca elliptica*	Annonaceae
11	Sp 3		
12	Sp 4		
13	Bụp lá nhỏ	*Hibiscus vitifolius*	Malvaceae
14	Sp		
15	Sp		
16	Sp		
17	Trường	*Xerospermum noronhianum*	Sapindaceae
18	Sp 3		
19	sp (same No 14)		
20	Săng mây	*Sageraca elliptica*	Annonaceae
21	Sp 3		
22	Lòng mang lá nhỏ	*Pterospermum grewiaefolium*	Sterculiaceae
23	Nhọc lá nhỏ	*Polyalthia cerasoides*	Annonaceae
24	Sổ	*Dillenia scabrella*	*Dilleniaceae*
25	Sp 3		
26	Sp 2		
27	Trường kẹn	*Mischocarpus sundaicus*	Sapindaceae
28	Nguyệt quế	*Murraya koenigii*	Rutaceae
29	Săng mây	*Sageraca elliptica*	Annonaceae
30	Thị hasselt	*Diospyros hasseltii*	Ebenaceae
31	Nhọc	*Polyalthia viridis*	Annonaceae
32	Máu chó lá nhỏ	*Knema globularia*	Myristycaceae
33	Làu táu	*Vatica odorata*	Dipterocarpaceae
34	Sp		Myrtaceae
35	Cọc rào	*Alphonsea gaudichaudiana*	Annonaceae
36	Thị đen	*Diospyros apiculata*	Ebenaceae
37	Sp 6		
38	Nhọc	*Polyalthia viridis*	Annonaceae
39	Sp 8		
40	Sp (same No 14)		
41	Sp		
42	Nguyệt quế	*Murraya koenigii*	Rutaceae

43	Nhọc	*Polyalthia viridis*	Annonaceae
44	Cọc rào	*Alphonsea gaudichaudiana*	Annonaceae
45	Công chúa lá nhỏ	*Artabotrys intermedius*	Annonaceae
46	Dâu da	*Baccaurea ramiflora*	Euphorbiaceae
47	Sp 11		
48	Sp		Annonaceae
49	Săng mây	*Sageraca elliptica*	Annonaceae
50	Sp 12		
51	Dâu da	*Baccaurea ramiflora*	Euphorbiaceae
52	Sp		Annonaceae
53	Dâu da	*Baccaurea ramiflora*	Euphorbiaceae
54	Nhọc lá nhỏ	*Polyalthia cerasoides*	Annonaceae
55	Sp		
56	Dâu da	*Baccaurea ramiflora*	Euphorbiaceae
57	Nhọc lá nhỏ	*Polyalthia cerasoides*	Annonaceae
58	Mò cua	*Alstonia scholaris*	Apocynaceae
59	Dâu da	*Baccaurea ramiflora*	Euphorbiaceae
60	Thị	*Diospyros sp.*	Ebenaceae
61	Thị	*Diospyros sp.*	Ebenaceae
62	Nhọc lá nhỏ	*Polyalthia cerasoides*	Annonaceae
63	Trường kẹn	*Mischocarpus sundaicus*	Sapindaceae
64	Nhọc	*Polyalthia viridis*	Annonaceae
65	Tung	*Tetrameles nudiflora*	Datiscaceace
66	Trường kẹn	*Mischocarpus sundaicus*	Sapindaceae
67	Sp		Annonaceae
68	Cồng Tía	*Calophyllum calata*	Clusiaceae
69	Sp		
70	Bằng Lăng Nước	*Lagerstroemia speciosa*	Lythraceae
71	Nhọc lá nhỏ	*Polyalthia cerasoides*	Annonaceae
72	Nguyệt quế	*Murraya koenigii*	Rutaceae
73	Thị hasselt	*Diospyros hasseltii*	Ebenaceae
74	Nhọc lá nhỏ	*Polyalthia cerasoides*	Annonaceae
75	Sp (same No 14)		
76	Sp		
77	Nhọc	*Polyalthia viridis*	Annonaceae
78	Dâu da	*Baccaurea ramiflora*	Euphorbiaceae
79	Dâu da	*Baccaurea ramiflora*	Euphorbiaceae
80	Nhọ nồi	*Diospyros apiculata*	Ebenaceae
81	Trường	*Xerospermum noronhianum*	Sapindaceae
82	Nhọc	*Polyalthia viridis*	Annonaceae
83	Nhọc	*Polyalthia viridis*	Annonaceae
84	Cọc rào	*Alphonsea gaudichaudiana*	Annonaceae
85	Lòng mang lá nhỏ	*Pterospermum grewiaefolium*	Sterculiaceae
86	Sp		
87	Dầu con rái	*Dipterocarpus alatus*	Dipterocarpace ace
88	Sp		Ebenaceae
89	Săng mây	*Sageraca elliptica*	Annonaceae

90	Dâu da	*Baccaurea ramiflora*	Euphorbiaceae
91	Nhọc	*Polyalthia viridis*	Annonaceae
92	Sp		
93	Nhọc lá nhỏ	*Polyalthia cerasoides*	Annonaceae
94	Dâu da	*Baccaurea ramiflora*	Euphorbiaceae
95	Săng mây	*Sageraca elliptica*	Annonaceae
96	Dâu da	*Baccaurea ramiflora*	Euphorbiaceae
97	Sp		Sterculiaceae
98	Sp (không thấy tán, vỏ hờm)		
99	Săng mây	*Sageraca elliptica*	Annonaceae
100	Thị đen	*Diospyros apiculata*	Ebenaceae

Appendix 3. Trees located on the Ben Cu transect. Unidentified trees are labeled as Sp.

Ben Cu Transect			
Sample #	Species: Vietnamese	*Species: Genus species*	
1	Trôm quạt	*Sterculia hypochrea*	Sterculiaceae
2	Bằng lăng xoan	*Lagerstroemia ovalfolia*	Lythraceae
3	Bằng lăng xoan	*Lagerstroemia ovalfolia*	Lythraceae
4	Cọc rào	*Alphonsea gaudichaudiana*	Annonaceae
5	Cẩm lai	*Dalbergia dongnaiensis*	Papilionoideae
6	Bằng lăng xoan	*Lagerstroemia ovalfolia*	Lythraceae
7	Bằng lăng xoan	*Lagerstroemia ovalfolia*	Lythraceae
8	Bằng lăng xoan	*Lagerstroemia ovalfolia*	Lythraceae
9	Bằng lăng xoan	*Lagerstroemia ovalfolia*	Lythraceae
10	Sp		
11	Bằng lăng xoan	*Lagerstroemia ovalfolia*	Lythraceae
12	Cẩm lai	*Dalbergia dongnaiensis*	Papilionoideae
13	Nhọc	*Polyalthia viridis*	Annonaceae
14	Bằng lăng xoan	*Lagerstroemia ovalfolia*	Lythraceae
15	Lòng mang	*Pterospermum .sp.*	Sterculiaceae
16	Sp		
17	Lòng mang	*Pterospermum .sp.*	Sterculiaceae
18	Bằng lăng xoan	*Lagerstroemia ovalfolia*	Lythraceae
19	co ke	*Grewia tometosa*	Tiliaceae
20	Sp		
21	Trau tráu	*Ochrocarpus siamensis*	Clusiaceae
22	Bằng lăng xoan	*Lagerstroemia ovalfolia*	Lythraceae
23	Nhọc lá nhỏ	*Polyalthia cerasoides*	Annonaceae
24	Ô môi	*Cassia agnes*	*Caesalpininoideae*
25	Sp		
26	Nhọ nồi	*Diospyros apiculata*	Ebenaceae
27	Bằng lăng xoan	*Lagerstroemia ovalfolia*	Lythraceae
28	Gáo tròn	*Haldina cordifolia*	Rubiaceae
29	Gáo tròn	*Haldina cordifolia*	Rubiaceae
30	Gáo tròn	*Haldina cordifolia*	Rubiaceae
31	co ke	*Grewia tometosa*	Tiliaceae
32	Bằng lăng xoan	*Lagerstroemia ovalfolia*	Lythraceae
33	Sp		
34	Duối ô rô	*Streblus ilicifolia*	Moraceae
35	Sp		
36	Sp		
37	Cọc rào	*Alphonsea gaudichaudiana*	Annonaceae
38	Gõ đỏ	*Afzelia xylocarpa*	Caesalpininoideae
39	Bằng lăng xoan	*Lagerstroemia ovalfolia*	Lythraceae
40	Bằng lăng xoan	*Lagerstroemia ovalfolia*	Lythraceae
41	Duối ô rô	*Streblus ilicifolia*	Moraceae
42	Bằng lăng xoan	*Lagerstroemia ovalfolia*	Lythraceae
43	Bằng lăng xoan	*Lagerstroemia ovalfolia*	Lythraceae
44	Cuống vàng	*Gonocaryum lobbianum*	Icacinaceae

45	Sp		
46	Mắt cáo	*Vitex tripinnata*	Verbenaceae
47	Chiếc tam lang	*Barringtonia macrostachya*	Lecythidaceae
48	Mắt cáo	*Vitex tripinnata*	Verbenaceae
49	Sp		
50	Cọc rào	*Alphonsea gaudichaudiana*	Annonaceae
51	Sp		
52	Lòng mang	*Pterospermum .sp.*	Sterculiaceae
53	Lòng mang	*Pterospermum .sp.*	Sterculiaceae
54	Lòng mang	*Pterospermum .sp.*	Sterculiaceae
55	Chai Thorel	*Shorea thorelii*	Dipterocarpaceae
56	Mận rừng	*Syzygium semarangense*	Myrtaceae
57	Lòng mang	*Pterospermum .sp.*	Sterculiaceae
58	Bằng lăng xoan	*Lagerstroemia ovalfolia*	Lythraceae
59	Sp		
60	Sp		
61	Sp		
62	Công chúa lá rộng	*Cacanga latifolia*	Annonaceae
63	Sp		
64	Lòng mang	*Pterospermum .sp.*	Sterculiaceae
65	Nhọc lá nhỏ	*Polyalthia cerasoides*	Annonaceae
66	Sp		
67	Cò ke	*Grewia tomentosa*	Tiliaceae
68	Sp		
69	Cọc rào	*Alphonsea gaudichaudiana*	Annonaceae
70	Lòng mang	*Pterospermum .sp.*	Sterculiaceae
71	Sp		
72	Sp		
73	Sp		
74	Thị	*Diospyros sp.*	Ebenaceae
75	Trau tráu	*Ochrocarpus siamensis*	Clusiaceae
76	Cẩm lai	*Dalbergia dongnaiensis*	Papilionoideae
77	Công chúa lá rộng	*Cacanga latifolia*	Annonaceae
78	Gõ đỏ	*Afzelia xylocarpa*	Caesalpininoideae
79	Trau tráu	*Ochrocarpus siamensis*	Clusiaceae
80	Sp		
81	Cẩm lai	*Dalbergia dongnaiensis*	Papilionoideae
82	Công chúa lá rộng	*Cacanga latifolia*	Annonaceae
83	Cẩm lai	*Dalbergia dongnaiensis*	Papilionoideae
84	Nhọ nồi	*Diospyros apiculata*	Ebenaceae
85	Công chúa lá rộng	*Cacanga latifolia*	Annonaceae
86	Cò ke	*Grewia tomentosa*	Tiliaceae
87	Sp		
88	Thị	*Diospyros sp.*	Ebenaceae
89	Bứa	*Garcinia sp.*	Clusiaceae
90	Sp		
91	Trau tráu	*Ochrocarpus siamensis*	Clusiaceae
92	Sp		
93	Bứa	*Garcinia sp.*	Clusiaceae

94	Sp		
95	Thị	*Diospyros sp.*	Ebenaceae
96	Sp		
97	Tung	*Tetrameles nudiflora*	Datiscaceace
98	Bằng lăng nước	*Lagerstroemia speciosa*	Lythraceae

Appendix 4. Trees located on the Da Co transect. Unidentified trees are labeled as Sp.

Da Co Transect			
Sample #	Species: Vietnamese	*Species: Genus species*	Family
1	Bằng lăng xoan	*Lagerstroemia ovalfolia*	Lythraceae
2	Cẩm lai	*Dalbergia dongnaiensis*	Papilionoideae
3	Sp		
4	Bằng lăng xoan	*Lagerstroemia ovalfolia*	Lythraceae
5	Nhọc lá nhỏ	*Polyalthia cerasoides*	Annonaceae
6	Chiêu liêu nước	*Terminalia calamansanai*	Combretaceae
7	Tung	*Tetrameles nudiflora*	Datiscaceace
8	Băng lăng xoan	*Lagerstroemia ovalfolia*	Lythraceae
9	Cọc rào	*Alphonsea gaudichaudiana*	Annonaceae
10	Công chúa lá rộng	*Cacanga latifolia*	Annonaceae
11	Máu chó lá nhỏ	*Knema globularia*	Myristycaceae
12	Sp2		
13	Nhọ nồi	*Diospyros apiculata*	Ebenaceae
14	Sp		
15	Hợp hoan	*Albizia sp.*	Mimosoideae
16	Sp 1		
17	Sp 2		
18	Sp 3		
19	Máu chó lá nhỏ	*Knema globularia*	Myristycaceae
20	Sp 4		
21	Nhọ nồi	*Diospyros apiculata*	Ebenaceae
22	Sp		Ebenaceae
23	Cồng	*Calophyllum ceriferum*	Clusiaceae
24	Quế rừng	*Cinnamomum polyadelphum*	Lauraceae
25	Sp		Ebenaceae
26	Mắt cáo	*Vitex tripinnata*	Verbenaceae
27	Chiếc tam lang	*Barringtonia macrostachya*	Lecythidaceae
28	Sp		
29	Máu chó lá nhỏ	*Knema globularia*	Myristycaceae
30	Dầu con rái	*Dipterocarpus alatus*	Dipterocarpaceace
31	Sp		
32	Thành ngạnh	*Cratoxylon maingayi*	Clusiaceae
33	Dâu da	*Baccaurea ramiflora*	Euphorbiaceae
34	Máu chó lá nhỏ	*Knema globularia*	Myristycaceae
35	Sp		Ebenaceae
36	Cẩm lai	*Dalbergia dongnaiensis*	Papilionoideae
37	Sp		Lauraceae
38	Bằng lăng xoan	*Lagerstroemia ovalfolia*	Lythraceae
39	Chiêu liêu nước	*Terminalia calamansanai*	Combretaceae
40	Dầu con rái	*Dipterocarpus alatus*	Dipterocarpaceace
41	Chiêu liêu nước	*Terminalia calamansanai*	Combretaceae
42	Sp5		
43	Sp1		
44	Xoài rừng	*Mangifera minutifolia*	Anacardiaceae

45	Tung	*Tetrameles nudiflora*	Datiscaceace
46	Tung	*Tetrameles nudiflora*	Datiscaceace
47	Dầu con rái	*Dipterocarpus alatus*	Dipterocarpaceace
48	Nguyệt quế	*Murraya koenigii*	Rutaceae
49	Tung	*Tetrameles nudiflora*	Datiscaceace
50	Chàm ron	*Colona evecta*	Tiliaceae
51	Sp		
52	Dâu da	*Baccaurea ramiflora*	Euphorbiaceae
53	Tung	*Tetrameles nudiflora*	Datiscaceace
54	Dầu con rái	*Dipterocarpus alatus*	Dipterocarpaceace
55	Nguyệt quế	*Murraya koenigii*	Rutaceae
56	Mận rừng	*Syzygium semarangense*	Myrtaceae
57	Sp		
58	Mắt cáo	*Vitex tripinnata*	Verbenaceae
59	Sp		
60	Sp		
61	Sp		Ebenaceae
62	Sp2		
63	Sp3		
64	Lòng mang	*Pterospermum .sp.*	Sterculiaceae
65	Sổ	*Dillenia scabrella*	*Dilleniaceae*
66	Bằng lăng xoan	*Lagerstroemia ovalfolia*	Lythraceae
67	Thành ngạnh	*Cratoxylon maingayi*	Clusiaceae
68	Thành ngạnh dẹp	*Cratoxylon formosum*	Clusiaceae
69	Bằng lăng xoan	*Lagerstroemia ovalfolia*	Lythraceae
70	Thành ngạnh dẹp	*Cratoxylon formosum*	Clusiaceae
71	Bằng lăng xoan	*Lagerstroemia ovalfolia*	Lythraceae
72	Thiên tuế	*Cycas rumphii*	Cycadaceae
73	Nhọ nồi	*Diospyros apiculata*	Ebenaceae
74	Bằng lăng xoan	*Lagerstroemia ovalfolia*	Lythraceae
75	Sp		Annonaceae
76	Nhọ nồi	*Diospyros apiculata*	Ebenaceae
77	Dầu con rái	*Dipterocarpus alatus*	Dipterocarpaceace
78	Sp		Annonaceae
79	Sp 3		
80	Bằng lăng xoan	*Lagerstroemia ovalfolia*	Lythraceae
81	Trau tráu	*Ochrocarpus siamensis*	Clusiaceae
82	Bằng lăng xoan	*Lagerstroemia ovalfolia*	Lythraceae
83	Sp		Annonaceae
84	Bằng lăng xoan	*Lagerstroemia ovalfolia*	Lythraceae
85	Bằng lăng xoan	*Lagerstroemia ovalfolia*	Lythraceae
86	Bằng lăng xoan	*Lagerstroemia ovalfolia*	Lythraceae
87	Bằng lăng xoan	*Lagerstroemia ovalfolia*	Lythraceae
88	Dầu lá bóng	*Dipterocarpus turbinatus*	Dipterocarpaceace
89	Sp		Ebenaceae
90	Bằng lăng xoan	*Lagerstroemia ovalfolia*	Lythraceae
91	Bằng lăng xoan	*Lagerstroemia ovalfolia*	Lythraceae
92	Mắt cáo	*Vitex tripinnata*	Verbenaceae
93	Bằng lăng xoan	*Lagerstroemia ovalfolia*	Lythraceae

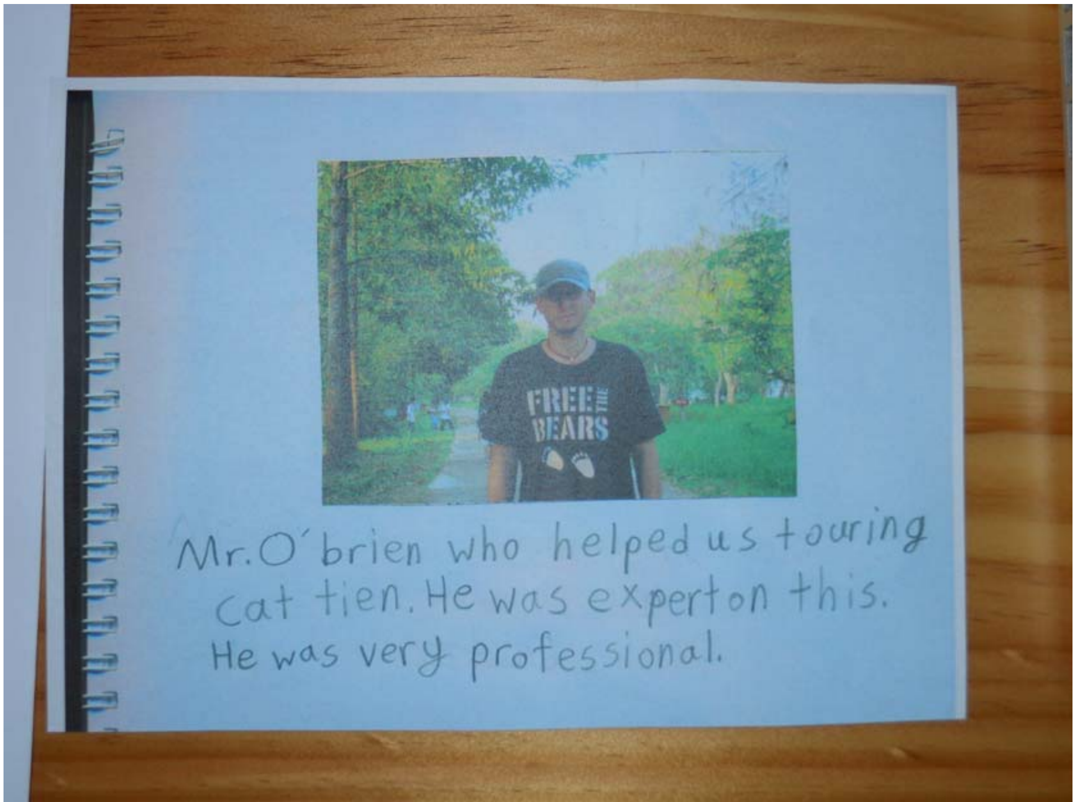
94	Trường kẹn	*Mischocarpus sundaicus*	Sapindaceae
95	Dâu da	*Baccaurea ramiflora*	Euphorbiaceae
96	Bằng lăng xoan	*Lagerstroemia ovalfolia*	Lythraceae
97	Sp 2		
98	Chiêu liêu nước	*Terminalia calamansanai*	Combretaceae
99	Nhọc	*Polyalthia viridis*	Annonaceae
100	Mắt cáo	*Vitex tripinnata*	Verbenacea
101	Công chúa lá nhỏ	*Artabotrys intermedius*	Annonaceae
102	Dâu da	*Baccaurea ramiflora*	Euphorbiaceae
103	Công chúa lá nhỏ	*Artabotrys intermedius*	Annonaceae
104	Mắt cáo	*Vitex tripinnata*	Verbenacea
105	Bằng lăng xoan	*Lagerstroemia ovalfolia*	Lythraceae
106	Bằng lăng xoan	*Lagerstroemia ovalfolia*	Lythraceae
107	Bằng lăng xoan	*Lagerstroemia ovalfolia*	Lythraceae
108	Sp		

Transect	Nutrient	Fisher's Exact Test (two sided p)	Lambda Asymmetric CIR
BS	N	**p<.001**	0.7692
BS	P	p<.0423	0.000
BS	K	p<.7775	0.0417
DC	N	**p<.0001**	0.3478
DC	P	p<.8591	0.000
DC	K	**p<.0331**	0.2069
BC	N	p<.0846	0.1333
BC	P	p = 1	0.000
BC	K	p = 1	0.000
ALL	N	**p<.001**	0.5143
ALL	P	p<.5266	0.000
ALL	K	p<.1561	0.0606

Appendix 5. Results of soil chemical analysis

Parasites Present	**Number of Samples**
Strongyloides, Trichuris	2
Strongyloides, Tapeworm	6
Trichuris, Tapeworm	1
Strongyloides, Trichuris, Tapeworm	5
Strongyloides, Pinworm	2
Strongyloides, Trichuris, Tapeworm, Mite	1
Strongyloides, louse	1
Strongyloides, *Trichuris*, oocyst	1
Strongyloides, mite	1
Strongyloides, Tapeworm, Pinworm	2
Tapeworm, mite	1
Tapeworm, nematode	1
Strongyloides, Tapeworm, tick	1
Tapeworm, pinworm	1
Strongyloides, Tapeworm, unknown	1
Strongyloides, *Trichuris*, Tapeworm, unknown	1
Total	**28**

Appendix 6. Parasites present in each sample



Appendix 7. Saigon South International School 5th grade student's opinion.